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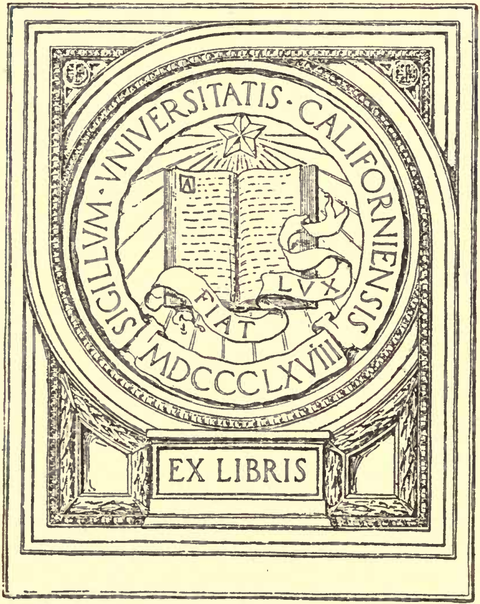
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A MANUAL
OF
BACTERIOLOGY

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BY

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REVISED BY

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WITH 113 ILLUSTRATIONS

FIFTH EDITION, REVISED AND ENLARGED

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PREFACE TO THE FIFTH EDITION.

In the preface to the second edition of this manual the author states that: "the purpose of this book is to give in the smallest possible space the facts which a physician must know, with some of those which it is desirable that he should know, and a little of that which he may learn if his needs or inclinations lead him to go further." While in the present edition this purpose has been kept in mind by the reviser, nevertheless it has been necessary to make many alterations in order to place the work abreast of the progress which has been made since the publication of the last edition. Some things which have become obsolete have been omitted, much that is new has been added, and the scope has been somewhat widened.

Since the book is designed primarily for medical students, the method of expression, as in former editions, is often more definite than is perhaps justifiable, and it must be understood that this form is adopted merely in order to make a clear presentation of the subject, not that the matter is in every such case finally settled, nor that the views as stated are necessarily shared by the author or the reviser, but that they are held by those whose opinions are worthy of consideration, and are the interpretation of the results of painstaking investigation, but which, nevertheless, may be in time modified or abandoned as a result of further research.

Those who are familiar with the former editions of the book will find that the arrangement of chapters is the same as heretofore. Many references to the literature have been added, but these are by no means exhaustive, many had to be omitted with regret. The hygienic examination of milk has been practically rewritten; the hygienic examination of water considerably expanded. The chapter on disinfectants, and that on surgical antisepsis have been also greatly altered. The interesting theoretical subject of the diversion of complement has been added to the chapter on immunity. The *trypanosomes* and the *amœbæ* have been given additional space, but certainly not more than these increasingly important microorganisms demand. The index has been not only greatly enlarged, but the references have been made much fuller and more specific.

A very full card catalogue of references prepared by Dr. Williams was made use of in looking up most of the articles which have been cited.

As in the fourth edition, the author is relieved of all responsibility for the statements made in the present edition; both for those retained from former editions, as well as for those which have been added by the reviser.

B. MEADE BOLTON.

WASHINGTON, D. C.,

PREFACE TO THE THIRD EDITION.

The plan used in the preceding editions of this manual has been followed in the preparation of the present one. The only departures have been in the insertion of a short historical sketch and the freer use of references to original articles and reviews. It is hoped that these features will assist in arousing the interest of students. As far as possible, reference has been made to articles in American and English journals likely to be easy of access. Besides the ones just named, numerous additions have been made which the recent advances in our knowledge have rendered necessary. Most of the illustrations of apparatus are new. The photomicrographs also are new and original, with a few exceptions noted in the text. It is probably needless to say that none of them were retouched. The writer is indebted to the Gratwick Laboratory, of Buffalo, for the use of its facilities in making these photographs.
H. U. W.

BUFFALO, NEW YORK, *August*, 1903.

CONTENTS.

PAGE
Introduction with Historical Sketch 1

PART I.

BACTERIOLOGICAL TECHNIC.

CHAPTER I.

Examination of Bacteria with the Microscope, Including Methods of Staining 18

CHAPTER II.

Sterilization 53

CHAPTER III.

Culture-media 64

CHAPTER IV.

The Cultivation of Bacteria. Tube-cultures; the Incubator; Anaerobic Methods. 78

CHAPTER V.

The Cultivation of Bacteria (Continued). Isolation of Bacteria; Plate-cultures 94

CHAPTER VI.

Inoculation of Animals. Autopsies; Collodion Sacs 102

CHAPTER VII.

Collection of Material 107

CHAPTER VIII.

Systematic Study of Species of Bacteria. Suggestions for Class-work; Rules 112

PART II.

EXPERIMENTAL STUDY AND PRACTICAL APPLICATIONS.

CHAPTER I.

PAGE

Classification; General Morphology and Physiology of Bacteria 117

CHAPTER II.

Products of the Growth of Bacteria 128

CHAPTER III.

Bacteria of Soil, Air, Water and of Milk and Other Foods 135

CHAPTER IV.

Bacteria of the Normal Human Body 160

CHAPTER V.

Bacteria in Disease 167

CHAPTER VI.

Bacterial Poisons; Agglutinins; Lysins; Precipitins 186

CHAPTER VII.

Immunity; Phagocytosis; Antitoxin 198

CHAPTER VIII.

Disinfection, Sterilization and Antisepsis 238

CHAPTER IX.

Surgical Antisepsis 262

PART III.

NON-PATHOGENIC BACTERIA 267

PART IV.

PATHOGENIC BACTERIA 282

PATHOGENIC PROTOZOA 407

APPENDIX 426

INDEX 438

LIST OF ILLUSTRATIONS.

FIG. PAGE

1. Micrococci, Bacilli, Spirilla 4
2. Test-tube with Culture-medium 7
3. Microscope 19
4. Abbé Condenser 20
5. Platinum Wires 22
6. Hanging-drop 23
7. Cornet Forceps for Cover-glasses 26
8. Stewart Forceps for Cover-glasses 26
9. Kirkbride Forceps for Slides 27
10. Schanze Microtome 39
11. Hot-air Sterilizer 54
12. Arnold Steam Sterilizer 57
13. Massachusetts Board of Health Sterilizer 53
14. Koch Steam Sterilizer 59
15. Autoclave 61
16. Kitasato Filter 62
17. Test-tube with Potato 72
18. Wire Basket for Test-tubes 77
19. Manner of Holding Test-tubes 79
20. Stab-culture 80
21. Smear-culture 80
22. Incubator 83
23. Reichert Gas-regulator 84
24. Gas-regulator, mercurial 84
25. Roux Bimetallic Gas-regulator 85
26. Koch Automatic Gas-burner 86
27. Rogers' Electric Thermo-regulator 88
28. Buchner's Method for Cultivating Anaerobes 90
29. Fränkel's Method for Cultivating Anaerobes 91
30. Novy's Method for Cultivating Anaerobes 92
31. Streak Culture of the Potato Bacillus 93
32. Petri Dish 96
33. Dilution-cultures in Esmarch Roll-tubes. 97
34. Appearance of Colonies on Gelatin in a Petri Dish 98

FIG. PAGE
35. Esmarch's Roll-tube 99
36. Mouse-holder 102
37. Apparatus for the Subcutaneous Insertion of Solid Substances . . . 103
38. McCrae's Method for Making Collodion Capsules 106
39. Cover-glass Preparation of Blood 108
40. Sternberg Bulb 109
41. Micrococci of Various Forms 118
42. Bacilli of Various Forms 119
43. Spirilla of Various Forms 119
44. Involution Forms 120
45. Bacteria with Capsules 122
46. Bacteria with Spores 123
47. Bacteria Showing Flagella 124
48. Fermentation-tube 132
49. Sedgwick-Tucker Aerobioscope 138
50. Receptors of the First Order Uniting with Toxin 219
51. Receptors of the Second Order and of Some Substance Uniting with One of Them 220
52. Receptors of the Third Order and of Some Substance Uniting with One of Them 222
53. "Spectrum" of Theoretically Fresh Crude Toxin 224
54. "Spectrum" of Very Fresh Crude Toxin 225
55. "Spectrum" of Crude Toxin as it is Supposed Always Practically to Occur 225
56. Mechanism of Bacteriolysis 230
57. Diversion of Complement in Undiluted Immune Serum 233
58. Partial Bacteriolysis, etc. 234
59. Bacillus subtilis 272
60. Spirilla from Swamp Water 275
61. Spirilla from Swamp Water with Flagella 276
62. Yeast Cells 278
63. Penicillium glaucum, Oïdium lactis, Aspergillus glaucus, Mucor mucedo 279
64. Staphylococcus pyogenes aureus in Pus 289
65. Staphylococcus pyogenes aureus in Pure Culture 291
66. Staphylococcus pyogenes aureus in Culture in Gelatin 299
67. Streptococcus pyogenes, Pure Culture 296
68. Streptococcus pyogenes in Pus 297
69. Streptococcus pyogenes Culture on Agar 298
70. Micrococcus tetragenus in Pus 299
71. Micrococcus lanceolatus (of Pneumonia) in Sputum 304
72. Micrococcus lanceolatus (of Pneumonia) Showing Capsules 305
73. Diplococcus intracellularis meningitidis 309

FIG. PAGE
74. Gonococcus in Pus 312
75. Bacillus pyocyaneus 318
76. Bacillus of Bubonic Plague 320
77. Bacillus aerogenes capsulatus. 324
78. Bacillus aerogenes capsulatus Culture. 326
79. Bacillus of Tetanus. 328
80. Bacillus of Anthrax. 331
81. Bacillus of Anthrax with Spores 332
82. Bacillus of Anthrax Colony 333
83. Bacillus of Anthrax Culture 334
84. Bacillus of Anthrax Showing Concave Ends 335
85. Bacillus of Anthrax in the Liver 336
86. Bacillus of Diphtheria. 339
87. Bacillus of Diphtheria, Neisser's Stain 340
88. Tubes for Cultivation of Diphtheria Bacillus. 340
89. Bacillus of Diphtheria, Culture. 341
90. Bacillus tuberculosis 350
91. Branching Form of Tubercle Bacillus 351
92. Bacillus tuberculosis, stained, in Sputum. 352
93. Ray-fungus of Actinomycosis, Fresh Preparation 367
94. Actinomyces bovis from a Pure Culture 369
95. Bacillus of Typhoid Fever. 370
96. Bacillus of Typhoid Fever with Flagella. 371
97. Widal Serum-reaction with Typhoid Bacilli 376
98. Bacillus coli communis 382
99. Bacillus coli communis with Flagella 383
100. Spirillum of Cholera 388
101. Spirillum of Cholera Involution Forms 391
102. Spirillum of Cholera Colonies on Gelatin Plates 392
103. Spirillum of Cholera Culture in Gelatin 393
104. Vibrio proteus 401
105. Spirillum of Relapsing Fever. 403
106. Malarial Parasite. 417
107. Malarial Parasite. 417
108. Malarial Parasite. 417
109. Malarial Parasite. 417
110. Trypanosomes in the Blood of the Rat 421
111. Plates for Counting Colonies. 440, 441, 442

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INTRODUCTION.

BACTERIOLOGY is not a subject which one should attempt to learn from books alone or without instructors; for aside from the difficulty or impossibility of such a task, there may be danger as well. Indeed the warning should perhaps be given in the beginning that in view of the fact that so many bacteria cause diseases which not only affect the individual who suffers, but makes him a menace to those about him, no one is justified in entering upon the study without proper guidance.

Anyone who has not himself worked in a bacteriological laboratory finds it difficult to form a vivid conception of what bacteria are like, because among the familiar animals and plants there are none with which a close comparison can be made. Of the common organisms, perhaps ordinary yeasts and moulds are most like the bacteria. Yeasts and moulds, as everyone knows, grow on bread, cheese, meat, syrups and the like. They flourish in moist and dark places, as do mushrooms, puffballs and the other fungi. All these fungi, appearing so different in some respects, are alike in one particular, which is the absence of the green color that we are apt to think of as being the essential feature of vegetation. Plants that are green owe their color to a substance called chlorophyll. Upon the properties of this substance one of the most fundamental vital phenomena in biology depends. By means of chlorophyll, under the influence of sunlight, plants are able to use as food the carbon dioxide which is always present in the atmosphere in small amounts. Although carbon dioxide is one of the simplest and most stable of compounds, its component elements are dissociated by the plant, and employed in the formation of other much more complex and unstable compounds, such as starch and cellulose, which enter into the plant's structure. The work of plants, it will be noticed, is in the main, precisely the reverse of that performed by animals. Animals take the unstable carbohydrates with high potential energy, such as starches and sugars, as food, and exhale the stable carbon dioxide from the lungs. At the same time the animal receives the benefit of the energy resulting from the oxidation of the carbohydrates, which may appear indirectly in the form of nervous or muscular activity or warmth.

Those plants that are devoid of chlorophyll are compelled to some extent to use the same kinds of food as animals. They are unable to decompose carbon dioxide (in most cases), and procure their nourishment from substances derived from the dead or living bodies of other plants or animals. Since they have no chlorophyll, light is of no advantage to them, and is often a positive detriment. Bacteria contain no chlorophyll, and consequently are unable to decompose carbon dioxide and to use it as food.*

There is another well-known property, possessed by yeasts especially, which may be useful in explaining the work done by bacteria. It is a fact of every-day observation that alcohol and gas are formed when ordinary yeast grows in fruit juice or other fluids containing sugar. It not only appears that bacteria sometimes form alcohol and gas from sugar, but that with different kinds of bacteria and different kinds of food material a great number of substances are formed, some of which are powerful poisons. In most, if not in all, of the diseases caused by bacteria such poisons are produced within the living body of the affected individual, and the symptoms of the disease and the changes produced in the body are certainly due to these poisons, as a rule, rather than to the direct action of bacteria.

On account of their extremely minute size, the bacteria cannot be seen as individuals without the aid of the microscope, although great numbers of them taken together may form a plainly visible mass of growth. When they are examined with the microscope they appear as little, round, rod-shaped or curved bodies, which may be likened to so many periods, dashes and commas. It is at once perceived that each bacterium is an individual by itself, and that it consists of a single cell, not of an aggregation of cells, as do most of the common plants and animals.

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* See Part II., Chapter I.

Under favorable conditions bacteria undergo rapid multiplication. The individuals in some forms divide into two cells, in other forms into four cells, in others again into eight cells simultaneously. The process takes place by direct cell division, and is called *fission*.

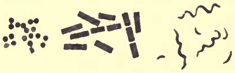
Under certain conditions, bright, glistening bodies make their appearance in certain bacteria, and become larger and larger, while the cells in which they develop break up into fine fragments. These bodies are called *spores*, and represent a resting stage in some respects resembling the seeds of higher plants. They have much greater resisting power against injurious influences than is possessed by the growing or *vegetative forms*. There are spores that can withstand boiling for hours, but fortunately that it not true of the spores of any of the bacteria that produce disease, as far as is yet known. The earlier investigators observed the appearance of bacteria in nutrient infusions which they had endeavored to sterilize by heat. They looked upon this fact as indicating the possibility of *spontaneous generation*, and it furnished the chief support of that theory. Probably their infusions contained very resistant spores, and were in reality not sterile.

Definition.—From these facts a definition for bacteria may be formulated.

Bacteria (Greek βαχτήριον, meaning a little stick) *are extremely minute, unicellular plants, which have no chlorophyll, and* *which divide by fission.* They are sometimes called *schizomycetes.* In every-day language they are known as *microbes* and also as *germs.* They are generally classed with the fungi. In some respects they seem quite closely related to the algæ or simplest green plants, and, on the other hand, they have strong points of likeness with some of the unicellular animals belonging to the infusoria.

Bacteria are divided into two great groups:

I. The lower bacteria include those forms which are of most importance at present, from a medical and economic point of view, and consist of:



Micrococci. Bacilli. Spirilla.

FIG. 1.

Cocco-bacilli—mostly short, thick, oval forms which also occur in rods.

Micrococci, or cocci (singular, coccus)—spherical forms.

Bacilli (sing., bacillus)—long and straight, or rod-shaped bacteria.

Spirilla (sing., spirillum)—consisting of spiral filaments like the turns of a corkscrew, or parts of spirals shaped like commas.

II. The higher bacteria, which consist of long filaments made up of segments more or less united. In some of these the filaments show dichotomous branching. This group is more fully discussed under the non-pathogenic bacteria, Part III. A few of them are pathogenic.

The extreme smallness of the bacteria is hard of comprehension. We may say, of most of them, that from 5,000 to 25,000 placed end to end would make a line about an inch in length. The tiny speck which adheres to the end of a fine platinum wire when this is used to obtain preparations from cultures is found upon examination with the microscope to consist of many thousands of bacteria.

It is well known that bacteria are present on most of the objects about us. They occur on the skins of men and other animals as well as in the mouth, stomach and intestines, and on most of the surfaces of the body that open to the external world. They are found in the water of rivers and lakes and in the ocean. They appear in the soil down to a depth of several feet. They float in the air, except at high altitudes and over the ocean. Nansen found bacteria on the ice of the Polar sea. Investigators have even reported finding them fossilized, indicating, as we might expect, that they existed at remote periods in the earth's history. But the vast majority of them are entirely harmless so far as we are concerned, and many of them are indispensable in maintaining the balance existing between dead matter and living beings.

Were it not for the putrefactive and nitrifying bacteria, the dead bodies of plants and animals would lie practically unchanged where they fall, and the fertilization of the soil necessary for the life of most plants, by means of substances derived from such dead material, would cease.

Some kinds of bacteria are useful in taking nitrogen from the atmosphere, and making it available as plant food in the soil, and are thus employed in the place of chemical fertilizers. Many of them have been made to subserve a useful purpose in the ripening of cream and cheese, and in the manufacture of vinegar from wine and cider. It has been suggested with some plausibility that anaerobic bacteria played an important part in the formation of coal from vegetable substances.

The ripening of ensilage in silos is a process of fermentation caused by bacteria.

In northern Siberia the bodies of the extinct species of elephant called mammoths have been found imbedded in frozen soil where they appear to have lain for thousands of years. In this case the growth of putrefactive bacteria has been prevented by cold, as in the modern refrigerator or cold-storage plant.

The study of bacteria has led to the understanding of many hitherto unexplained phenomena. The unaccountable development of a moist, brilliant red deposit on bread and other articles of food, which was formerly believed by the superstitious to be blood, deposited by some miraculous agency, we know to be due to the growth of a common organism (bacillus prodigiosus). The emission of light by decaying substances when seen in the dark is caused by bacteria as well as other organisms.

It seemed that in some cases in which death has been attributed to the suction of air into the veins, because air appeared to be present inside the heart, the air was in reality a gas formed by certain bacilli that invaded the body just before or just after death (bacillus aërogenes capsulatus).

Woodhead tells us that some savages are in the habit of smearing the soil of certain localities upon their arrows for an arrowpoison, which is intelligible in the light of the fact that soil often contains the bacilli of tetanus (lockjaw).

The comparatively small number of species of bacteria that cause disease are the ones that interest us most, and are those which have been most carefully studied. Since the bacteria in common with other fungi are compelled to derive their food from organic matter, it is easy to understand that they should frequently exist as parasites upon living animals and plants. Pear-blight and some other diseases of plants are caused by bacteria. Bees and other insects, frogs, birds, cattle and a great number of animals besides men suffer from diseases produced by bacteria.

When bacteria are placed upon slips of glass they may be studied with the microscope while alive. Some of them when living are motionless; others wriggle vigorously. Some dart about like minnows in a stream, or they make their way slowly across the field of the microscope like a boat that is being sculled from the stern. By proper methods it can be shown that the movements are effected through one or more fine, hair-like processes called flagella.

Often it is expedient to study bacteria after drying them on slips of glass, when they may be made more conspicuous by giving them an artificial color (staining). Some of the substances of which they are composed readily absorb certain dyes. For this purpose the aniline dyes are used, and their employment has been one of the important factors in making progress in bacteriology possible.

With the microscope alone it is not usually practicable to distinguish accurately between various kinds of bacteria. Micrococci, for instance, which are, in reality, entirely different species, may look very much alike. But differences usually become apparent when the bacteria are grown artificially. The cultivation is done for the most part in test-tubes containing some material which furnishes suitable food. The nutrient materials most used are meat-extract and peptone, which, dissolved with salt in water, constitute *nutrient bouillon.* Ordinary *gelatin,* or a vegetable gelatin called *agar-agar,* may be added to the bouillon when a solid *culture-medium* is desired. Before these substances can be used for the cultivation of bacteria all other bacteria which they may contain must be destroyed by heat.

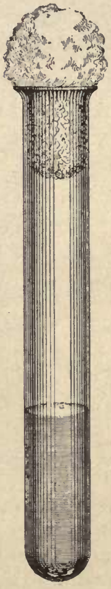


FIG. 2.—Test-tube containing culture-medium.

Finally, the effects of bacteria in bringing about disease may be tested on the lower animals. The proof that a particular species of bacteria causes a particular disease cannot be considered complete unless the disease can be reproduced by introducing these bacteria into some animal.

Bacteriological Literature.—The student who wishes to pursue bacteriological study in any direction farther than it is possible for the limits of a short manual to go, may, besides consulting the large text-books and weekly medical journals, obtain much assistance from technical periodicals. The *Journal of Experimental Medicine*, *Journal of Medical Research*, and the *Journal of Infectious Diseases*, published in this country, and the English *Journal of Pathology and Bacteriology* and *Journal of Hygiene* devote much valuable space to the subject.

The *Journal of Tropical Medicine* also contains contributions.

A reading knowledge of German and French is very desirable. The *Centralblatt für Bakteriologie*, etc., a German periodical, and the *Bulletin de l'Institut Pasteur*, published semimonthly in Paris, contain abstracts of most of the important researches made in all parts of the world. The *Annales de l'Institut Pasteur*, the *Zeitschrift für Hygiene*, the *Archiv für Hygiene* and the *Hygienische Rundschau* contain many original articles on bacteriological subjects.

The whole literature of any specified subject in bacteriology can be most conveniently found in *Baumgarten's Jahresbericht der Mikroorganismenlehre*.

Those who are interested in agricultural bacteriology should read the experiment station records and the various bulletins issued by the Department of Agriculture of the United States. They can usually be obtained upon application to the Department at Washington, D. C. The bacteria that produce disease in domestic animals are described in Dr. V. A. Moore's book, "The Infectious Diseases of Animals," Taylor & Carpenter, Ithaca, N. Y., 1906, and in the "Special Report on the Diseases of Cattle," United States Department of Agriculture, 1904; also Diseases of the Horse, Department of Agriculture, 1907

Historical Sketch.—Nearly all that we know of bacteria and the part they play in producing disease has been learned during the last half of the last century. Nevertheless, many facts were known long ago, and even by the ancients, which were effective in directing the thought of later years. The epidemic nature of certain maladies was naturally among the earliest of these to be noticed, and was, even until comparatively recent times, attributed to the influence of gods, demons or other supernatural agencies. The superstitions and crude beliefs of the past gave rise to a mass of grotesque theories and fanciful speculations. But with all this we hear of certain beliefs and practices which plainly foreshadowed those of the present day. Latin writers nearly two thousand years ago recorded a relation between insects and malaria which has but lately been proved and explained. The isolation of lepers by the ancient Hebrews shows that the infectious character of the disease has long been recognized, though other affections than leprosy were probably confused with this disease by the ancients. "He is unclean: he shall dwell alone; without the camp shall his habitation be" (Lev. XIII., 46). There is, in fact, much in the laws of Moses that points to some knowledge of the nature of infections. "This is the law, when a man dieth in a tent: all that come into the tent and all that is in the tent shall be unclean for seven days. And every open vessel which has no covering upon it shall be unclean" (Numb. XIX., 14, 15).

"Everything that may abide the fire, ye shall make it go through the fire, and it shall be clean" (Numb. XXXI., 23).

In Homer we read of Ulysses, that, having slain his wife's troublesome suitors:

> "With fire and sulphur, cure of noxious fumes,
> He purged the walls and blood-polluted rooms" (Pope's Odyssey).

The massive aqueducts of the Romans still remain to testify that they understood the importance of a pure water-supply.

In Rome there were also sewers for the disposal of drainage, while the Cretans and Assyrians used sewerage systems hundreds and even thousands of years before.

About the fourteenth century we find quarantine against infectious diseases, plague in particular, practiced by certain Italian cities; and the word "quarantine" came into use from the fact that the period of detention was about forty days (Ital. *quarantina*).*

Leeuwenhoek, a citizen of Delft, in Holland (1632–1723) appears to have been the first who actually saw bacteria. Yeast-cells he certainly observed, besides making many other contributions of great value to biology. Leeuwenhoek produced admirable lenses of high magnifying power, and described what he witnessed with singular accuracy and enthusiasm.

Even before this time men had sought to explain the phenomena of infectious diseases by supposing the body to have been penetrated by minute parasites—for example, worms. The spread of such diseases through a community from a single center could readily be accounted for by the multiplication of a contagious element, itself alive (*contagium vivum*). With increasing knowledge of the abundance of microscopic life these speculations took firmer hold. But long before their truth was finally demonstrated great advances were made in the prevention of infectious diseases. Much honor is due the clinicians, whose accurate observations and foresight accomplished important results at an early day, working with what now seems a very meagre knowledge of the facts.

The production of immunity from small-pox by inoculation was first practiced in oriental countries. The method had long been in use in the East, when in 1718 it was brought to the notice of Europeans by Lady Montagu, wife of the English ambassador at Constantinople. The procedure consisted simply of the introduction of the virus of small-pox by puncture of the skin. An attack of small-pox resulted, which was usually much milder and far less dangerous than the natural disease.

Lady Montagu stated in a letter: "Every year thousands

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*J. M. Eager. The Early History of Quarantine. *Yellow Fever Institute Bulletin*, No. 12. U. S. Marine Hospital Service.

undergo the operation; and the French ambassador says pleasantly that they take the small-pox here by way of diversion, as they take the waters in other countries." The mild attacks that followed inoculation were, however, just as contagious to other persons as the natural disease, so that the dangers of this practice to the community were very great.

This was previous to the introducion of vaccination by Edward Jenner in 1796. At this time a belief was current among farmers that a mild form of disease, called cow-pox, acquired by milkers, furnished protection against small-pox, and on investigation Jenner found this belief to be justified. In a few years the practice of vaccination spread to all parts of the world.*

It was introduced into the United States by Dr. Benjamin Waterhouse, of Harvard. President Thomas Jefferson was active in bringing it into general use, especially in the South.

The infectious nature of puerperal fever was first demonstrated by Semmelweis, of Vienna, in 1847. Before this time unsuccessful attempts had been made to prove that atmospheric influences were responsible for the disease, and during the seventeenth and eighteenth centuries the course had been supposed to lie in the absorption of the milk from the breasts into the blood. But Semmelweis was struck by the similarity between puerperal fever and a fatal case of pyemia following a dissecting wound in the case of a friend of his, and was led by this observation to attribute the origin of the disease to poisons carried on the fingers of physicians and students from the dissecting-room to the woman in childbed. This idea of Semmelweis aroused opposition and ridicule, but it withstood these tests though the originator somewhat modified his views. As a prophylactic measure, Semmelweis advocated

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*See the works of Edward Jenner by Dock. *New York Medical Journal* Nov. 29 and Dec. 6, 1902. Also Dulles. The History of Vaccination. *Philadelphia Medical Journal.* May 30, 1903.

washing the hands of the attendant at child-birth in solutions of chlorine or chloride of lime in addition to cleansing them with soap and water.

During the same period similar ideas were advanced by Dr. Oliver Wendell Holmes in the United States. His paper on "The Contagiousness of Puerperal Fever" appeaed in 1843. A lively controversy lasting several years was provoked, in which Holmes defended his position with great vigor. His admirable literary style served him effectively.*

In the first half of the nineteenth century, with improved microscopes, knowledge of minute living things grew rapidly, chiefly with respect to infusoria and other relatively large forms. In 1840 Henle described the part played by microorganisms in producing disease in terms surprisingly in accord with views held at the present time. His deductions were based almost entirely on knowledge of the general nature, spread and course of infections. So, too, Villemin anticipated the discovery of the bacillus of tuberculosis, for he transmitted the disease to animals, by inoculating them with material from cases of tuberculosis in man.

The key to exact knowledge of the microörganisms of disease was finally discovered in the study of fermentation. No better illustration could be found of the possible value to mankind which may lie in any addition whatever to the common stock of knowledge. The study of bottles of bad-smelling broth would have seemed, fifty years ago, a most unpromising beginning for the discovery of the causes of cholera, plague and the like, or for an antitoxin for diphtheria.

Studies on Fermentation and Spontaneous Generation.—Two observers (Schwann, Cagniard-Latour, 1837) almost simultaneously advanced the idea that yeast-cells were living organisms, and that the fermentation of solutions of sugar was due to their growth. This aroused a controversy which lasted more than thirty years. Prominent among those who contended against the agency of living cells in the production of fermentation was Liebig, but in spite of this the doctrine steadily gained ground, and was extended to include other sorts of fermentation and the putrefaction of albuminous material. Different kinds of fermentation, with different products, such as acetic acid and butyric acid, were eventually shown to be due to the growth of different kinds of microbes.

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* See O. W. Holmes. Medical Essays. Houghton, Mifflin & Co., 1889.

These microbes were found to be fungi of various sorts, and chiefly one or another variety of bacteria. The most celebrated among the students of fermentation was Pasteur, the simplicity and kindness of whose character excite our admiration equally with his devotion to his work.*

Before the nature of fermentation was understood, the possibility of spontaneous generation had been universally admitted. When vermin of various sorts appeared in putrefying material the conclusion was drawn that they had their origin directly from it. Although that had long since been disproved in the case of large organisms like worms and frogs, still, as late as the middle of the last century, it was held by many to account for the swarming microscopic life found in fermenting fluids. A flask of meat broth left exposed to the air will after a few days contain countless tiny living things, chiefly bacteria. Pasteur and his followers showed that these bacteria were the progeny of others already in the flask or which had fallen in from the air.

When the flask of broth was boiled, no development of organisms took place, if the entrance of germs from the atmosphere was prevented. The latter was accomplished by such devices as heating the air, passing it through sulphuric acid, using a flask with a long twisted neck or by plugging the flask with cotton (Schröder and Von Dusch).

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*See Louis Pasteur. His Life and Labors. By His Son-in-Law. Translated by Lady Claude Hamilton.

To prove that boiling had not made the fluid unfit for the growth of organisms, air was subsequently allowed to have access to it without such precautions, when putrefaction took place in the usual manner.

These principles underlie the methods used daily for the preservation of meat, fruit and vegetables, in the household and in canning factories.

Although boiling occasionally failed to prevent fermentation, investigators came with practice to have a smaller number of failures. Such failures it was shown were due to the presence of the resistant forms of the organisms called spores previously alluded to which some bacteria assume. The true nature of spores was recognized later by Cohn. Pasteur found that exposure to steam at temperatures sufficiently high above the boiling point would destroy the most resistant microbes and their spores. But even boiling and subsequent protection from the entrance of bacteria sometimes met with failure.

The controversies over fermentation and putrefaction lasted almost until the present day. They have been productive of numerous benefits to the arts and manufactures. But, what is of more importance to our subject, they led to a vastly better understanding of diseases produced by microorganisms. The study of bacteria has been pursued with such vigor in the last thirty-five years in fact that most of what we know concerning the bacteria of disease has been learned during this period, and advances are still constantly being made.

The discussions concerning fermentation and putrefaction were still going on when Lister made his brilliant deduction that suppuration and septic processes in wounds were a species of fermentation (1867). From this came the antiseptic and aseptic methods of operating and of dressing wounds, which have made possible the wonderful results of modern operative surgery.*

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*See Roswell Park. History of Medicine.

In 1834 the parasite of itch (*Acarus scabiei*, the itch mite, an arachnid, related to the insects) was discovered, and the cause of one contagious malady determined.

Quite early in the nineteenth century also the relatively large fungi of thrush and some of the parasitic skin diseases were discovered. The bacilli of anthrax, which are also relatively large, were seen in the blood of animals by Pollender in 1855 and Davaine in 1863.

Davaine produced anthrax in animals by injecting into them blood containing anthrax bacilli. But complete proof that these bacilli were the cause of the disease required that they should produce it when injected alone and when freed from the smallest trace of material derived from the first diseased animal. Unless these conditions were complied with, some other material, for example an enzyme or ferment, might be supposed to be carried from the first to the second animal and to be the real cause of the disease. For this purpose it was necessary to cultivate the bacilli outside the animal body in an artificial medium of some kind, such as meat broth, as was done by Pasteur. It then became possible to demonstrate that their properties could remain unaltered after being grown in successive generations on different lots of broth. As bacteria of two or three species were often encountered in mixtures, it became most important to secure a method by which the different species could be separated from one another and be propagated as separate "pure cultures." This was done successfully by diluting such mixtures greatly, so that a drop planted in a new tube of broth should contain only a single organism. The growth ensuing would of course consist of the same kind of organism exclusively. Such procedures were uncertain and very laborious.

Koch introduced in 1881 his method of separating bacteria by "plating," described below (Part I., Chapter V.), and this is probably the most important contribution to bacteriological technic which has ever been made. Koch also pointed out the advantages of solid media for the propagation of pure cultures. Other important technical improvements of the same period were the adoption of the illuminating apparatus of Abbé and immersion objectives, and of aniline dyes for staining bacteria and making them visible (Weigert and Ehrlich). Beginning with the bacillus tuberculosis described by Koch in 1882, a number of pathogenic bacteria were discovered during the ensuing years in rapid succession.

The application of the newly-gained knowledge concerning the bacteria causing infectious diseases to the prevention and cure of these diseases was begun almost immediately by Pasteur. A few facts existed to guide the direction of the research. It had been known even in ancient times that one attack of an infectious disease, such as scarlet fever, may confer immunity from subsequent attacks.

The protection against small-pox which was furnished by vaccination also was suggestive, although the mechanism by which this protection came about was not understood.

Pasteur worked on the theory that immunity from a disease would probably be secured by producing a mild attack of the disease. Such a mild attack might be expected to follow if a susceptible individual were inoculated with microbes of lowered virulence. Various methods were employed to reduce the virulence of bacteria, notably cultivation at high temperatures (43°C.). Pasteur was able to produce immunity against a number of the diseases of the lower animals. His method of inoculating sheep and cattle against anthrax has been employed with some success. A somewhat similar principle has led to the preparation of a vaccine for the disease of cattle called "black leg," and such vaccine is now distributed gratuitously to farmers by the United States government. Inoculation of human subjects with the attenuated virus is used for hydrophobia. This method also was devised by Pasteur.

But not only have inoculations against the microörganisms themselves been perfected in some cases, but also the substances which are called antitoxins, the specific agents against the poisonous products of the bacteria, have been produced. The discovery of these antitoxins for infectious diseases (see Part II., Chapter VII.) we owe to Behring. This portion of our subject belongs entirely to the present day, and is now being studied with great energy.

Allusion has already been made to moulds and other microscopic parasites whose nature makes their study almost inseparable from that of the bacteria. In this class also belong the primitive forms of animal life, the Protozoa, which are the causes of amebic dysentery (Lösch, 1875) and malaria (Laveran, 1880). The disease of cattle called "Texas fever" is also caused by a protozoön. Theobald Smith in the United States discovered that the parasite of Texas fever is conveyed from one animal to another by the cattle-tick. Since then it has been shown (by Manson, Ross and others) that malaria is conveyed by mosquitoes from a person having the disease to one not affected. It has also been shown by Reed and Carroll that a similar relation exists between mosquitoes and yellow fever. The part played by flies and other insects in carrying disease germs is still receiving active attention and the future may show that these play a most important part in diseases other than those already mentioned'.

In 1903 Novy and McNeil succeeded in making pure cultures of pathogenic protozoa grow in tubes, in nearly the same way that cultures of bacteria are propagated (see Appendix).

It is encouraging to reflect that the progress of bacteriology has been made by gradual and logical steps. The great discoveries have not been lucky accidents, but have been worked out patiently and with deliberation.

PART I.

CHAPTER I.

EXAMINATION OF BACTERIA WITH THE MICROSCOPE, INCLUDING METHODS OF STAINING.

The Microscope.—The microscope consists of a tubular body which carries the optical parts, and which can be raised or lowered for focusing. It is a matter of convenience to have three lenses attached to the body of the instrument by means of a triple nose-piece, which permits any objective to be turned into the optical axis at will. But a low-power dry lens and an oil-immersion objective are all that are essential for studying the bacteria. The eye-piece slips into the upper and opposite end of the body or tube. The arrangements for focusing consist of a rack and pinion which accomplish the coarse adjustment, and a more delicate fine adjustment. The stage, upon which the objects to be examined are placed, has an opening in the middle. In this opening an iris diaphragm and Abbé condenser are inserted. The iris diaphragm enables one to alter the size of the opening as desired. Beneath the stage is a movable mirror, of which one side is plane and the other concave. All of these parts are supported on a short, heavy pillar, which is fixed in the horseshoes-shaped base.

The essential parts of the microscope are, of course, the eye-piece (German, *Ocular*) and the objective. Objectives are variously designated by different makers, for instance, some use letters, A, B, C, etc., others use numbers, 1, 2, 3, etc. others again give the focal distance, as $\frac{2}{3}$ inch, $\frac{1}{4}$ inch, $\frac{1}{2}$ inch, etc. In bacteriological work a rather "low-power" $\frac{2}{3}$ or $\frac{3}{4}$-inch objective, and a high-power $\frac{1}{12}$ inch oil-immersion objective are needed. A $\frac{1}{4}$ or $\frac{1}{6}$ inch dry objective may also occasionally be useful. The magnification with the $\frac{2}{3}$ or $\frac{3}{4}$-inch objective is about 75 to 100 diameters; with the $\frac{1}{4}$ to $\frac{1}{6}$-inch 300 to 500 diameters; with the $\frac{1}{12}$ immersion 750 to 1000 diameters. The magnification varies according to the eye-piece used, as well

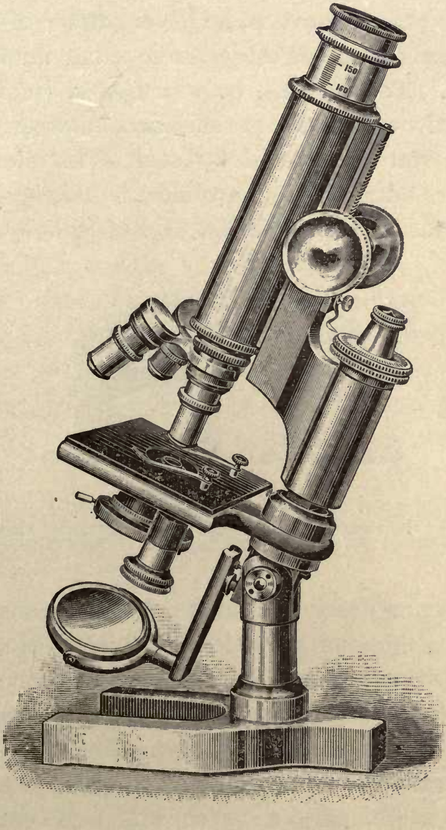

FIG. 3.—Microscope.

as with the objective. A 1-inch and $1\frac{1}{2}$-inch eye-piece (Zeiss No. 2 and No. 4) serve well for most purposes. The eye-pieces are usually named arbitrarily, like the objectives. In using the $\frac{1}{12}$ immersion objective a layer, of thickened oil of cedarwood is placed between the lower surface of the objective and the upper surface of the glass covering the object under examination. The oil must be wiped away from the surface of the objective when the examination is finished. For this purpose the soft paper sold by dealers in microscopic apparatus serves admirably. Care must be taken not to scratch the lower surface of this objective. Oil of cedar-wood furnishes a medium having nearly the same refractive index as the glass of the lens as the glass on which the object is mounted, and it obviates the dispersion of light which takes place when a layer of air is interposed between the objective and the object, as happens with the ordinary dry lens. This object is used in connection with the Abbé condenser, which consists of two or three lens combined so as to focus the rays coming from the plane mirror upon the object. The condenser gives a very intense illumination over a very small field. The condenser is not necessary excepting with the oil-immersion objective. If it is used with the other objectives, the illumination must be regulated by lowering the condenser, closing the diaphragm more or less, and substituting the concave for the plane mirror.

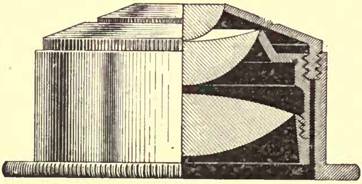

FIG. 4.—Abbé condenser.
On the right side the figure gives a sectional view.

It is to be remembered that more depends upon securing a distinct picture than upon a very high magnification of the object.

The microscope should be placed in front of the observer on a firm table with the light in front. The observer should be able to bring the eye easily over the eye-piece when the tube of the microscope is in vertical position. Daylight should be employed if possible, but not direct sunlight. When artificial illumination is necessary, an ordinary lamp, a Welsbach burner or an incandescent electric light may be used. It is best to modify the artificial light by inserting a sheet of blue glass between the light and the mirror.

In order to focus upon any object, having first secured a satisfactory illumination with the mirror, it is best, beginning with the low power and using the coarse adjustment for focusing, to bring the objective quite close to the object, and then, with the eye in position, to raise the tube until the object comes into focus. The exact focusing is done with the fine adjustment. The observer should keep both eyes open when using the microscope, and should be able to use either eye at will.

All measurements of microscopic objects are expressed in terms of a micromillimeter. This is one-thousandth of a millimeter (0.001 mm.), which is about $\frac{1}{25000}$ of an inch. It is generally called a micron for short, and is denoted by the Greek letter μ. For example, $5\ \mu = 0.005$ mm. $= \frac{1}{5000}$ inch.

The Preparation of Specimens of Bacteria for Examination with the Microscope.—The substance under examination is placed upon thin slips of glass called cover-glasses. The material is spread over the cover-glass by means of a platinum wire which has been fixed in a glass rod about six inches long. Such a platinum wire is used constantly in doing bacteriological work. It is the tool by means of which one is able to handle bacteria with impunity. The platinum

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wire must be stiff enough not to bend too easily, and yet it should not be so large that it will not cool rapidly after heating. A good size for most purposes is No. 23, American wire gauge (Brown & Sharp). The wire may be straight throughout its length, or the tip may be bent to form a loop (German, *Oese*). It is well to follow, from the beginning, certain rules which make the use of the platinum wire safe and accurate. Every time it is taken into the hand and before using it for any manipulation heat it in the flame of a Bunsen burner or an alcohol lamp to a red heat; and always, *after using*, and *before putting it down, heat it again to a red heat.* If the needle is wet it should be dried by holding it near the flame in order to avoid the "sputtering" which occurs if it is plunged at once into the flame. This precaution is especially called for when the wire has been dipped in milk or other substances containing oil. When the needle "sputters," as it is called, from too rapid heating, particles that have not yet been sterilized may be thrown some distance. On no account should the needle touch any object other than that which it is intended it should touch. With such a platinum wire, which has been properly sterilized, one can easily remove portions from a culture of bacteria, or from a fluid in which bacteria are supposed to be present. The glass rod in which the platinum wire is fixed should be held between the thumb and forefinger of the right hand like a pen. (For the manner of holding test-tubes, see page 79.)

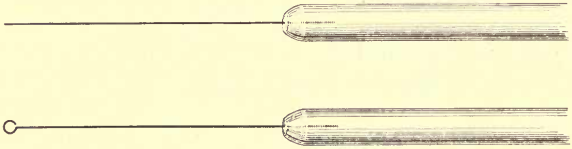

FIG. 5.—Straight platinum wire and platinum loop.

The Hanging-drop.—Living bacteria may be studied with the microscope while suspended in some fluid substance. This is accomplished by means of a hanging-drop. In order to prepare a hanging-drop for examination a clean cover-glass is held in the forceps and a small drop of the fluid to be examined is spread thinly over the center of it by means of a platinum needle which has just previously been heated in a flame and allowed to cool. The needle should again be sterilized in the flame. When cultures on solid media are to be examined, a small particle may be mixed with a drop of sterilized physiological salt solution or bouillon which has first been placed in the middle of the cover-glass. The cover-glass should have been carefully cleaned and sterilized over the flame. The cover-glass with the thin drop of fluid material held in sterilized forceps is now to be inverted over a sterilized glass slide, which has a concavity ground in the middle of it. Around the concavity, the slide should be smeared with vaseline. In this manner a small, air-tight chamber is made. This preparation may be put upon the stage of the microscope. A good dry lens, if of sufficiently high power, is more convenient for examining the hanging-drop than an oil-immersion. If the latter be used, having placed a drop of cedar-oil on the center of the cover-glass, and a good light having been secured, the oil-immersion objective should be brought down upon this drop of oil. The beginner often experiences difficulty in focusing upon a hanging-drop. It is well to shut off most of the light by means of the iris diaphragm. Often it is well to secure the focus roughly upon the extreme outer edge of the chamber, or to find the edge of the drop of fluid with the

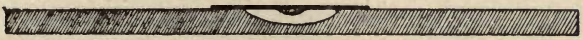

FIG. 6.—Diagram of the hanging-drop.

low power and then to focus upon this edge with the oil-immersion objective. Above all things guard against breaking the cover-glass by forcing the objective down upon it. The motility of certain bacteria is one of the most striking phenomena to be observed in the hanging-drop. It is not to be confused with the so-called "Brownian movement" which is exhibited by fine particles suspended in a watery fluid. It is well for the beginner to observe the character of the Brownian movement by rubbing up some dry, powdered carmine in a little water, and with the microscope to study the trembling motion exhibited by these particles of carmine. It will be noticed that, although the particles oscillate, no progress in any direction is accomplished unless there are currents in the fluid. Such currents might give rise to the impression that certain bacteria possessed motility when they were, in fact, powerless to move of themselves. In the hanging-drop the multiplication of bacteria can be studied, the formation of spores and the development of spores into fully formed bacteria. The hanging-drop has recently been put into service for the demonstration of the so-called serum-reaction with the bacillus of typhoid fever. Sometimes bacteria must be watched in the hanging-drop for hours, or even days, and it may be necessary to keep it at the temperature of the human body for this length of time. Various complicated kinds of apparatus have been devised for this purpose, but they are needful only with special kinds of work. When the hanging-drop preparation is no longer required, the slide and cover-glass should be dropped into a 5 per cent. carbolic acid solution and afterward sterilized by steam.

Hanging block preparations, which were introduced by Hill,* consist in the use of a cube of nutrient agar instead of a drop of fluid. Bacteria are distributed on the surface of the agar, which is then applied to a cover-glass, and mounted like

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**Journal of Medical Research.* Vol. VII. March, 1902.

a hanging-drop. The bacteria are kept in a layer close to the glass, where growth may be studied.

Cover-glass Preparations.—The study of bacteria with the microscope is for the most part done by means of smears made upon thin cover-glasses. It is best to obtain the kind sold by dealers as No. 1, ¾-inch squares.

The cover-glass may be cleaned best by immersion in a mixture of sulphuric acid and bichromate of potassium solution, and afterward washed thoroughly in distilled water, and finally in alcohol. A stock of clean cover-glasses may be kept in a bottle of alcohol, or perhaps preferably in alcohol containing 3 per cent. of hydrochloric acid. When they are needed for use they should be wiped clean with a piece of linen cloth. If they are heated at the time the preparations are made the bacteria will be found to spread more readily than on the cold surface and to adhere better. Whenever it is taken into the fingers it should be held by the edges, never by the flat surfaces. In spreading bacteria upon it and in all subsequent manipulations, as staining, the cover-glass should be handled with the forceps. It can be used very conveniently in the form of forceps known as the Cornet forceps, or in the modification devised by Stewart. Bacteria may be placed upon the cover-glass by allowing the glass to fall upon one of the colonies of bacteria, on a gelatin or agar plate (see page 98), which will adhere to it in part, producing an "impression preparation" (German, *Klatschpreparat*). Such a preparation, after drying in the air, is to be fixed by passing it through the flame three

CLEANING FLUID.

Potassium bichromate	40 grams.
Water	150 c.c.
Dissolve the bichromate of potassium in the water, with heat; allow it to cool; then add slowly and with care sulphuric acid, commercial	230 c.c.

times. (See below.) The forceps with which it is handled should be sterilized in the flame.

Generally bacteria contained in fluids, like sputum, or taken from the surface of a culture, are smeared over the cover-glass by means of the platinum wire or loop, which must be heated to a red heat before and after the operation. Such preparations are called smear, cover-glass, cover-slip, or film preparations. When the material to be spread is thick or very viscid, a small drop of distilled water must first be placed in the center of the cover-glass so as to dilute it. Beginners generally take too much material on the wire. As thin a smear as possible is made. It is allowed to dry in the air; this should occupy a few seconds. The drying may be hastened by holding the forceps with the cover-glass a long distance above the flame, at a point where the heat would cause no discomfort to the hand. Having dried the preparation, it is to be passed with the smeared surface up three times through the flame of a Bunsen burner or alcohol lamp. This should not be done too slowly and yet sufficiently so to fix the preparation. Various directions are given by different authors as to the time which

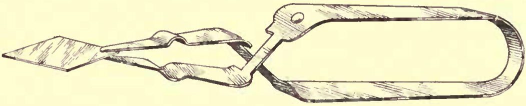

FIG. 7.—Cornet forceps for cover-glasses.

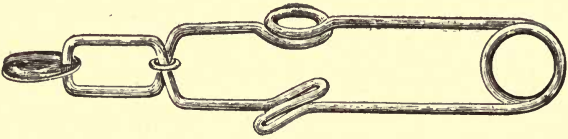

FIG. 8.—Stewart forceps for cover-glasses.

should be employed, but none of these appear exact, and one soon acquires by practice an idea as to how long the manipulation should take. Indeed, this varies with the character of the preparation. The heat of the flame serves to dry the bacteria upon the cover-glass and make them adhere permanently in position. It also aids in the penetration of the staining dyes; but it is not sufficient, when applied in this manner, to kill all kinds of bacteria, especially those containing spores. After it has been passed through the flame three times, the preparation may be stained with a solution of one of the aniline dyes, as described below, and after washing in water and drying may be mounted, face down, in Canada balsam upon a glass slide. It makes a suitable object to be examined with the oil-immersion objective.

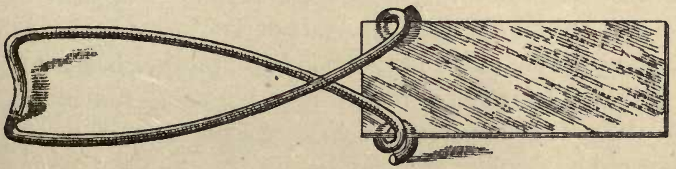

FIG. 9.—Kirkbride forceps for holding slides.

The smear preparation may equally well be made directly upon the glass slide. The fixation in the flame must then occupy a longer time than with the small and thin cover-glass. Such preparations have the advantage that several may be made upon one slide, and that after staining them they may be examined in cedar-oil, with the oil-immersion lens, without the use of the cover-glass and Canada balsam. The forceps of Kirkbride will be found convenient for staining on the slide. Experiments performed in the writer's laboratory have shown that the ordinary method of fixation in the flame, when applied to bacteria spread upon slides, has little effect on the vitality of many species. The beginner is, therefore, advised to make his preparations on cover-glasses.

When very resistant or dangerous pathogenic bacteria are being handled, after fixation by heat upon the slide or cover-glass, the preparation may, if desired, be immersed in 1–1000 solution of bichloride of mercury long enough to kill the bacteria, without injuring the preparation or interfering with its staining properties.

Staining.—The bacterial cells are devoid of color, and the object of staining them is to give them artificially some color that would make them distinct and easily visible with the microscope. In order that they shall stand out sharply the stain employed should leave the background unstained. This result is best obtained with aqueous solutions of the aniline dyes. These aniline dyes, so called, are derivatives of coal-tar, but not always of aniline. They are indispensable in bacteriological work. Their number is very large, but only a few are in common use. It is important to have the purest, and those manufactured by Grübler are reliable.

It is simplest to classify the aniline dyes as acid or basic. Eosin, picric acid and acid fuchsin are acid dyes; they tend to stain tissues diffusely. Fuchsin, gentian-violet and methylene-blue are basic dyes; they have an affinity for the nuclei of tissues and for bacteria; they therefore are the dyes used chiefly in bacteriological work. The other kinds may be employed as contrast-stains; another contrast-stain frequently used is Bismarck brown. It is best to keep on hand saturated solutions of the aniline dyes in alcohol, from which watery solutions may be made when needed by adding a few drops of the alcoholic solution to a small dish filled with water. The alcoholic solution is diluted about ten times, so as to make a liquid which is just transparent in a layer about 12 mm. in thickness, after filtering.

Fuchsin and gentian-violet operate rapidly and intensely.

Methylene-blue works more slowly and feebly; it is to be preferred where the bacteria occur in thick or viscid substances, like pus, mucus and milk, and acts more energetically when made slightly alkaline.

Method of Staining Cover-glass Preparations.—(*a*) A smear preparation of bacteria having been made, dried and passed through the flame three times in the manner above described, and a watery solution of either fuchsin, gentian-violet or methylene-blue having been prepared, the cover-glass is to be dropped into a dish containing the dye, or the dye may be dropped upon the cover-glass held in the forceps.

(*b*) Allow the stain to act for about thirty seconds.

(*c*) Wash in water.

(*d*) Examine with the microscope in water directly or after drying and mounting in Canada balsam.

The rapidity and intensity of staining may be increased by warming the solution slightly. The preparation may also be improved by rinsing for a few seconds in ½ per cent. acetic acid after staining. If this is done quickly, and the preparation thoroughly washed in water it does not materially affect the stain in the bacteria, and it clears up the background, thus bringing out the bacteria in stronger relief. The acid should be merely poured on and poured off, otherwise the bacteria will be more or less decolorized.

Preparations that are mounted at first in water may be made permanent by letting a drop of water fall at the edge of the cover-glass so that it may easily be removed from the slide, then drying and mounting in Canada balsam. Cover-glass preparations which have been stained are examined with oil-immersion objective, employing the plane mirror, having the iris diaphragm open and the condenser close to the lower surface of the glass slide. The purpose is to obtain the most intense illumination possible over a small field. The watery solutions of aniline dyes prepared as above described deteriorate in a short time, and it is best to prepare them freshly each time they are required. A very useful solution, which is permanent, is Löffler's alkaline methylene-blue:

Concentrated alcoholic solution of methylene-blue	30 c.c.
Potassium hydrate (caustic potash), 1–10,000 watery solution	100 c.c.

Löffler's methylene-blue is a good stain for general purposes. It is perhaps more in use than any other formula for coloring the diphtheria bacillus.

Aniline-water Staining Solutions.—The intensity with which aniline dyes operate may be increased by adding aniline oil to the solution:

Aniline oil	5 c.c.
Water	100 c.c.

Mix, shake vigorously, filter; the fluid after filtration should be perfectly clear; add—

Alcoholic solution of fuchsin (or gentian-violet, or methylene-blue)	11 c.c.
Alcohol	10 c.c.

Aniline-water staining solutions do not keep well, and need to be freshly prepared about every ten days or two weeks. The keeping quality depends probably upon the temperature and possibly on the exposure to light. Sometimes it keeps longer than at others, even when prepared each time alike. It is a good plan to filter it every time before use. Precipitates form during the first twenty-four hours after the stain is made, and for this reason cleaner preparations are obtained with the stain after it has stood for a day. The alcohol advised in the accompanying formula tends to dissolve the precipitate. Various other formulæ are given by different authors for the preparation of aniline-water staining solutions, but the one given above will be found to give satisfactory results. The applications of the aniline-water stains will be given under separate headings. In general, however, they are employed where a stain of unusual power is required.

Gram's Method.—The advantages of Gram's method are that by using it certain kinds of bacteria may be stained a violet color, while other bacteria are stained feebly or not at all. Cover-glass preparations, having been prepared preferably from agar slant cultures twelve to twenty-four hours old* and fixed in the usual manner (see pages 26 and 27), are stained as follows. Some advise short rinsing with water after pouring off the staining solution.

(*a*) Stain in aniline-water gentian-violet solution, from two to five minutes. The intensity of the stain may be increased by warming slightly.

(*b*) Iodine solution, one and one-half minutes:

Iodine	1 gram.
Potassium iodide	2 grams.
Water	300 c.c.

In this solution the preparation becomes nearly black.

(*c*) Wash repeatedly in strong, preferably in absolute alcohol; the alcohol becomes stained with clouds of violet coloring matter; the alcohol is used as long as the violet color continues to come away, and until the preparation is decolorized or has only a faint gray tint. It may be necessary to repeat the treatment with the iodine solution.

(*d*) When desired, the specimens may be stained, by way of contrast, with a watery solution of Bismarck brown or eosin.

(*e*) Wash in water, and examine either in water directly or after drying and mounting in Canada balsam. Gram's method and its modifications are not always trustworthy for diagnostic purposes; since one and the same organism may stain but faintly if all at one time, and quite intensely at others.† Still it is a more or less useful aid, particularly with practice. Bearing this qualification in mind, the following lists will serve as a guide.

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*Muir and Ritchie. American Ed., 1903, p. 103. Also Mallory and Wright. 1904, p. 101.

†Kolle and Wassermann. Vol. I, p. 70.

Bacteria that are stained by Gram's method:

Staphylococcus pyogenes aureus,
Streptococcus pyogenes,
Micrococcus lanceolatus (of pneumonia),
Micrococcus tetragenus,
Bacillus of diphtheria,
Bacillus of tuberculosis,
Bacillus of leprosy,
Bacillus of anthrax,
Bacillus of tetanus,
Bacillus aërogenes capsulatus,
Ray fungus of actinomycosis.

Of these the tubercle bacillus and the bacillus of leprosy require a much longer exposure to the stain than other bacteria in the list.

List of bacteria that are not stained by Gram's method:

Gonococcus,
Diplococcus intracellularis meningitidis,
Micrococcus melitensis,
Bacillus of chancroids (Ducrey),
Bacillus of dysentery (Shiga),
Bacillus of typhoid fever,
Bacillus coli communis,
Bacillus pyocyaneus,
Bacillus of influenza,
Bacillus of bubonic plague,
Bacillus of glanders (bacillus mallei),
Bacillus of Friedländer,
Bacillus proteus,
Spirillum of Asiatic cholera,
Spirillum of relapsing fever.

Staining the Bacillus of Tuberculosis.—Since the tubercle bacillus does not take the ordinary stains readily, a very large number of methods have been proposed for staining it, all of which depend upon the principle that, after adding to solutions of aniline dyes certain substances, like aniline-water, carbolic acid, or solutions of ammonia or soda, the bacillus tuberculosis is stained with great intensity, and gives up its stain with difficulty. Solutions of acids will remove the stain from all parts of the preparation excepting from the tubercle bacilli, which retain the dye, once having acquired it. The rest of the preparation may now be given a different color—contrast-stain.

Bacilli that resist decolorization by acids are called *acid-proof* or acid-fast. The most important are tubercle and leprosy bacilli. There are various other species, however, most of which are less resistant to acids and alcohol than tubercle bacilli. They are discussed in the article on the bacillus tuberculosis in Part IV.

Occasionally spores of other bacteria, micrococci and horny epithelial cells are imperfectly decolorized, but their forms distinguish them from tubercle bacilli. Minute crystalline needles which have a shape like that of bacilli are often encountered in sputum, but their nature will be recognized after a little practice.

The stain for tubercle bacilli is most frequently used for specimens of sputum from cases of suspected pulmonary tuberculosis; it may be applied to other fluids and secretions equally well. It is not reliable, however, when applied to milk, as the oil present in milk interferes with its operation, and milk and its products quite often contain other acid-proof bacilli. The smegma of the external genitals also frequently contains acid-proof bacilli that are not tubercle bacilli. On this account all fluids and discharges from the genito-urinary tract need to be examined with particular care not to confuse tubercle bacilli with smegma bacilli. (See smegma bacilli in Part II., Chapter IV.)

Patients should be given minute instructions concerning the collection of sputum. The bottle used should be new, wide-mouthed, clean, and kept tightly stoppered with a clean cork. The patient should be cautioned against allowing the expectoration to get on the outside of the bottle. Probably whatever risk is incurred by those who examine sputum comes chiefly from the outside of the bottle having been soiled with sputum containing tubercle bacilli. Often little white particles may be seen floating in the mucous portions of the sputum. These particles should be selected for the investigation, and may be spread in a thin film on the cover-glass with the platinum wire, which is sterilized in the flame before and after using. The selection of the little white particles will be facilitated if the sputum be poured into a clean glass dish, which may be placed on a black surface. A form of porcelain dish is furnished by dealers, the bottom of which is black, and which is convenient, for these manipulations. The smears must be made thin, or the subsequent decolorization, after staining, will not be uniform. It is hardly necessary to observe that the operator must be scrupulously careful not to contaminate the material under examination with any kind of extraneous matter. The cover-glasses and slides which are used should be new, and should have been cleaned with bichromate of potassium and sulphuric acid (see page 25).

When the work is completed, the bottle containing the sputum should be sterilized by steam or boiling.

Many different methods for staining the tubercle bacillus have been proposed. In most of those now in use the following solution (Ziehl's carbol-fuchsin) is employed—

Fuchsin	1 gram.
Carbolic acid, pure	5 c.c.
Alcohol	10 c.c.
Distilled water	100 c.c.

N. B.—Acid fuchsin cannot be used for this stain.

The method given below is the one recommended.

Method for staining the tubercle bacillus:

(*a*) The cover-glass preparation is made, dried and fixed by passing through the flames three times (see pages 26 and 27).

(*b*) The cover-glass, held in forceps or in a watch-crystal, is covered with carbol-fuchsin and heated till bubbles begin to appear showing that the water in the stain is boiling. The stain should be allowed to act for five minutes and kept hot during this time. It is not always necessary to heat as high as this nor to allow the stain to remain for so long a time; but in order to be sure in all cases it is best to do so.

(*c*) Wash in water.

(*d*) Wash in alcohol containing 3 per cent. of hydrochloric acid one minute, or longer if necessary to remove the red color.

(*e*) Wash in water.

(*f*) Stain with methylene-blue solution (see page 30) thirty seconds.

(*g*) Wash in water.

It may be found necessary to repeat the treatment with acid alcohol, and this should be done if the color returns to the preparation after washing in water.

(*h*) Examine in water directly, or after drying and mounting in Canada balsam. Tubercle bacilli take a brilliant red color; other bacteria and the nuclei of cells are stained blue.

Gabbett's Method.—This method is very popular and widely used on account of its convenience. It is not as reliable as the one just given.

Gabbett's solution:

Methylene-blue	1 to 2 grams.
25 per cent. watery solution of sulphuric acid	100 c.c.

(*a*) The cover-glass preparation is to be made, dried and fixed by passing through the flame three times.

(*b*) The carbol-fuchsin stain is applied from two to five minutes to the cover-glass, held in forceps or in a watch-crystal; it need not be warmed.

(*c*) Wash in water.

(*d*) Gabbett's solution is applied for one minute.

(*e*) Wash in water. The preparation should have a blue color. It may be examined in water directly or after drying and mounting in Canada balsam.

Gabbett's method has the advantage of decolorizing the preparation and staining the background with methylene-blue at the same time. Tubercle bacilli are colored a brilliant red; most other bacteria and the nuclei of cells are colored blue. The acid-proof bacilli mentioned on page 33 also retain the red stain in most cases, and might be confused with tubercle bacilli.

Of the numerous methods of staining tubercle bacilli, only a few others can be mentioned. Aniline-water fuchsin, aniline-water gentian-violet or carbol-fuchsin may be used. The intensity of the stain may then be increased by warming the preparation till it steams or boils, and allowing the warm stain to act on the specimens for from three to five minutes; the preparation may also be left in the cold stain over night. Decolorization of the background may be effected with a 25 per cent. solution of sulphuric acid used till the color disappears, or a 30 per cent. solution of nitric acid, which operates very rapidly. If the color persists after washing in water, it should be dipped in the acid again. After either acid the preparation is to be washed in alcohol until the last trace of the stain has been removed. An excellent decolorizing agent is a 3 per cent. solution of hydrochloric acid in alcohol, used for about a minute. With any of these acid solutions the decolorization can be accomplished more perfectly than with Gabbett's solution, where the operation of the decolorizing agent is masked. The contrast-stain may be omitted entirely if it is desired. A suitable contrast-stain after fuchsin staining is a solution of methylene-blue; after gentian-violet staining, Bismarck brown.

Those who have had experience in staining tubercle bacilli soon discover that the bacilli exhibit some differences in their resisting power to strong acids. One encounters occasionally bacilli that are perfectly stained side by side with others that are more or less completely decolorized. These facts show the necessity of practice with any method, and of exercising caution and judgment in making a diagnosis where the number of bacilli happens to be scanty. If tubercle bacilli are not found in the first preparation, other preparations should be made. Sometimes a large number of cover-glasses must be examined.

Various expedients have been devised to concentrate tubercle bacilli when only a small number may be present in a sample of sputum. In Biedert's method about 15 c.c. of sputum are mixed with 5 c.c. of distilled water, 4 to 8 drops of sodium hydrate solution are added, and the mixture is boiled. After boiling, about 15 c.c. of distilled water are added. The mixture may be set aside in a conical glass for from twenty-four to forty-eight hours to allow sedimentation or the sediment may be precipitated rapidly by the use of the centrifuge. In either case cover-glass preparations are made from the sediment in the manner already described. The sediment will be found to have little adhesive power, and will not stick well to the cover-glass. It is convenient to save some of the original sputum and mix it with the sediment for this purpose.

Fixation of Tissues.—Pieces of organs about 1 cm. in thickness may be taken. Alcohol is the best agent for preserving them. The fixation will be completed in a few days. It is best to change the alcohol. The amount of the alcohol must be twenty times the bulk of the tisuse to be preserved.

Ten parts of the standard 40 per cent. solution of formaldehyde, with 90 parts water make a good mixture for fixation; after twenty-four hours change to alcohol.

Imbedding in Collodion or Celloidin.—From alcohol the pieces of tissue are placed in equal parts of alcohol and ether, twenty-four hours; thin collodion (1½ per cent.), twenty-four hours; thick collodion of a syrupy consistency (6 per cent.), twenty-four hours. The specimen is laid upon a block of wood or, better, the compressed vegetable fibre called vulcanite, and surrounded by thick collodion, and then placed in 70 per cent. alcohol. The collodion makes a firm mass, surrounding and permeating the tissue, and permits very thin sections to be cut. The soluble cotton sold by dealers in photographers' supplies serves as well as the expensive preparation known as celloidin. To make collodion, dissolve it in equal parts of alcohol and ether. Soluble cotton is also called pyroxylin, and is a kind of gun-cotton.

Imbedding in Paraffin.—Pieces of tissue 2 to 3 mm. thick which have already been fixed in alcohol or formaldehyde are to be placed:

(*a*) In absolute alcohol for twenty-four hours.

(*b*) In pure xylol one to three hours.

(*c*) In a saturated solution of paraffin in xylol one to three hours.

(*d*) In melted paraffin having a melting-point of 50° C., which requires the use of a water-bath or oven, one to three hours. The xylol must be entirely driven off, and the tissue thoroughly infiltrated.

(*e*) In fresh paraffin for one hour.

The tissue is finally placed in a small dish or paper box and covered with the melted paraffin. The paraffin should be hardened as quickly as possible with running water. It is important to fix the piece of tissue in the desired position before pouring in the melted paraffin.

Paraffin imbedding is especially useful when serial sections re to be made. Sections of exquisite thinness are possible ith this form of imbedding. The knife need not be wet.

In order to mount the sections, proceed as follows:

(*a*) Place the sections on water in a porcelain capsule. Varm slightly, when the sections will flatten nicely. Smear he surface of a slide with a very thin layer of Mayer's glycerin-lbumen mixture. Dip the slide under the sections; raise it up, and drain off the water, leaving the sections adhering to the glass in their proper positions. Let them dry for some hours in the incubator, and they will be firmly fastened to the slide.

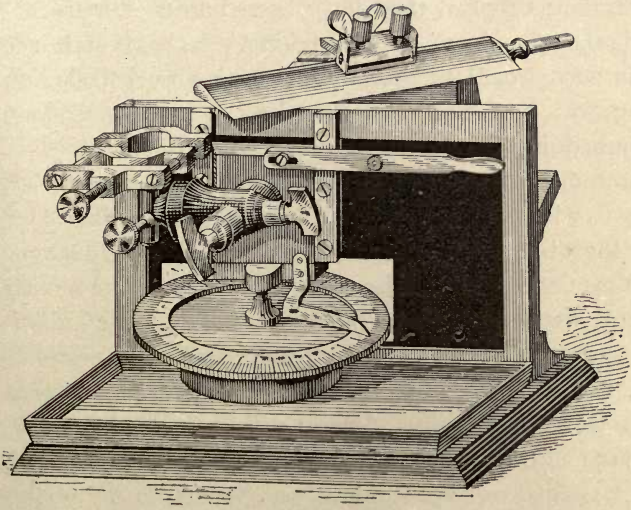

FIG. 10.—Schanze Microtome.

(*b*) Dissolve out the paraffin in one of the numerous solvents (xylol, a few minutes).

(*c*) Wash off the xylol with absolute alcohol, and

(*d*) Stain the sections as desired.

(*e*) Dehydrate in absolute alcohol.

(*f*) Clear in xylol.
(*g*) Mount in balsam.

GLYCERIN-ALBUMEN MIXTURE (MAYER).

Equal parts of white of egg and glycerin are thoroughly mixed, and then filtered. 'Add a little gum-camphor to preserve.

Section Cutting.—Cutting is best done with an instrument called a microtome. The tissues may be imbedded in collodion or paraffin; or when they have been hardened with formaldehyde they may be cut after freezing. Bacteria stain admirably in such frozen sections. For routine work collodion imbedding will be found as convenient a process as any. Paraffin imbedding gives the thinnest sections.

A microtome consists of a heavy, sliding knife-carrier, which moves with great precision on a level, and of a device for elevating the object which is to be cut any desired distance after each excursion of the knife. The thickness of the section will be the distance which the object is elevated. The knife is kept wet with alcohol during the cutting of collodion sections, otherwise it is left dry. The microtome is usually provided with a special form of knife. A razor will serve nearly as well, after having had the lower side ground flat. If a razor is used, a special form of razor-holder must be attached to the microtome to receive the razor. Above all, it is necessary that the knives should be kept in good condition. Only occasionally will they need honing, using a fine water-stone or Belgian hone. Preferably the knife should not be honed directly on the stone itself but on a piece of clean plate glass, on which the stone is first rubbed with water. By this means the entire cutting edge is sharpened in one plane. The movement in honing should be from heel to toe, and toward the cutting edge, always placing the back of the knife next the hone when turning. The knife should be stropped frequently. The leather of the strop should be glued to a strip of wood to make a flat surface. The movement in stropping should be from toe to heel. Sections should be cut to a thickness of not more than 25 μ. Thinner sections (5 to 10 μ) are to be desired.

Staining of Sections.—A watery solution of one of the basic aniline dyes is used—fuchsin, gentian-violet or methylene-blue—made by adding one part of the alcoholic solution to ten parts of distilled water. Löffer's solution of methylene-blue serves very well.

By this process most bacteria are stained; also the nuclei of cells; frequently, also, certain granules contained within some cells (German, *Mastzellen*), which may easily be mistaken for bacteria by the inexperienced (basophilic granules).

(*a*) Place the section in the staining solution from two to five minutes or longer.

(*b*) Wash in water.

(*c*) Place in a watery solution of acetic acid, $\frac{1}{10}$ per cent., for from a few seconds to one minute.

(*d*) Alcohol, one to two minutes; change to absolute alcohol. Touch the sections to blotting-paper to remove the superfluous alcohol.

(*e*) Xylol until clear; xylol is to be preferred to other clearing agents, like oil of cloves, most of which slowly remove aniline colors. It has the disadvantage of not clearing when the slightest trace of water is present; dehydration in alcohol must, therefore, be complete. The section should be removed from the xylol as soon as it is cleared; otherwise wrinkling occurs.

(*f*) The section is placed upon a glass slide; a drop of Canada balsam is placed upon it and then a cover-glass. The Canada balsam should be dissolved in xylol.

The section is to be manipulated with straight or bent needles. The removal from xylol to the glass slide is managed best with a spatula or section-lifter.

The above statements apply to frozen sections or to sections imbedded in celloidin. Paraffin sections are preferably attached to the slide with glycerin-albumen. The different steps in the process follow in the same order. The stain may be poured on the slide, or the slide may be placed in a large dish full of staining fluid. (See page 29.) Celloidin sections may also be stained on the slide. If the section be well spread and flattened thoroughly with blotting-paper, it will usually adhere to the slide, and is less likely to wrinkle. It must not be allowed to dry.

Gram's Method may be applied to the staining of sections of tissues as well as to smears upon cover-glasses.

(*a*) Place the section in aniline-water gentian-violet, five minutes or longer. See the preceding paragraph for the manner of handling sections.

(*b*) Rinse in water.

(*c*) Iodine solution (see page 31), one and one-half minutes.

(*d*) Alcohol, until decolorized to a faint blue-gray.

(*e*) Xylol.

(*f*) Mount on a slide in balsam.

Weigert's Modification of Gram's Method.—(*a*) Place the section in aniline-water gentian-violet solution, five minutes or more. See page 41 for the manner of handling sections.

(*b*) Rinse in water.

(*c*) After placing the section upon a slide, and having straightened it carefully, absorb the water with blotting-paper.

(*d*) Iodine solution (see page 31), one to two minutes.

(*e*) Absorb the iodine solution with blotting-paper.

(*f*) Add aniline oil, removing it from time to time with blotting-paper, and adding fresh aniline oil until the specimen becomes a faint blue-gray and until the color ceases to come away. (Aniline oil serves in this connection both to decolorize and to dehydrate. It absorbs the water rapidly and efficiently. However, on account of its decolorizing tendency, it must be removed before the specimens can be mounted permanently.)

(*g*) Wash in several changes of absolute alcohol.

(*h*) Add xylol; remove it with blotting-paper; and add fresh xylol several times, in order to extract the last trace of aniline oil.

(*i*) Mount in Canada balsam.

This method is more convenient for the staining of sections than the Gram method. The results, however, are essentially the same as far as the bacteria are concerned; fibrin is usually stained blue, hyaline material is also stained blue, and bacteria violet. It is often impossible to decolorize the nuclei completely without decolorizing the bacteria also. The parts of the nuclei which remain stained often present pictures that resemble bacteria, and which may lead to error if not recognized. Basophilic granules also retain the stain, as do the horny cells of the epidermis. These remarks apply also to Gram's method, except as regards fibrin. Very beautiful preparations can be obtained according to this or the Gram method when the sections have previously been stained in carmine; the nuclei will then be colored red, bacteria violet.

Where sections are stained with carmine they should be thoroughly washed before applying the Gram stain, since the presence of acid interferes with this stain,* and any of the acid alcohol which is used after the carmine must be carefully removed.

Tubercle bacilli may be *stained in sections* as follows:

(*a*) Use carbol-fuchsin, or aniline-water gentian-violet for one-half to two hours with very gentle warming, or over night without warming. See page 41 for the manner of handling sections.

(*b*) Wash in water.

(*c*) Decolorize with some one of the decolorizing agents mentioned in connection with the staining of tubercle bacilli in cover-glass preparations, preferably 3 per cent. hydrochloric acid alcohol. Decolorization must be continued until the red or violet color, as the case may be, has disappeared, which requires one to several minutes.

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*Kolle and Wassermann. Vol. I., p. 70.

(*d*) Wash thoroughly in alcohol.

(*e*) Wash in water.

(*f*) Use hematoxylin as a contrast-stain for fuchsin preparations, and carmine for gentian-violet preparations. In the latter case it is better to stain with carmine before staining the bacilli. The carmine is not affected by the subsequent treatment.

(*g*) Wash in water.

(*h*) Alcohol.

(*i*) Xylol.

(*j*) Balsam.

Tubercle bacilli may also be stained in sections by Gram's method; but they require to be stained in the gentian-violet aniline oil for a longer time than do other bacteria which are stained by this method. The same statement also applies to the leprosy bacilli when stained by Gram's method.

Nuclear stains, which may be used as contrast-stains for section:

DELAFIELD'S HEMATOXYLIN.

Hematoxylin crystals	4 grams.
Alcohol	25 c.c.
Ammonia alum	50 grams.
Water	400 c.c.
Glycerin	100 c.c.
Methyl-alcohol	100 c.c.

Dissolve the hematoxylin in the alcohol, and the ammonia alum in the water. Mix the two solutions. Let the mixture stand four or five days uncovered; it should have become a deep purple. Filter and add the glycerin and the methyl-alcohol. After it has become dark enough, filter again. Keep it a month or longer before using; the solution improves with age. At the time of using, filter and dilute with water as desired.

LITHIUM-CARMINE (ORTH).

Carmine	2.5 grams.
Saturated watery solution of lithium carbonate	100.0 c.c.

Bacteria occasionally stain with hematoxylin though as a rule but faintly. The stain is not used for bacteria but only for staining the nuclei of the tissue cells.

Add a few crystals of thymol. The carmine dissolves readily in the lithium carbonate solution. Filter the stain at the time of using. Sections are to be left in the stain five to twenty minutes.

Sections stained in carmine are placed directly in acid alcohol (1 part hydrochloric acid, 100 parts 70 per cent. alcohol) for five to ten minutes. They acquire a brilliant scarlet color. When used as a contrast-stain for tissues containing bacteria, it is best to use it before staining the bacteria, which might be decolorized by the acid alcohol. The sections should for this reason be thoroughly washed before applying the Gram stain.

Staining of Blood-films.—*Romanowsky Stain.*—There are various modifications of this stain. MacNeal* states that the following formula gives the best result for physicians and for clinical laboratories:

Prepare crude methylene violet by boiling for 15 minutes over a free flame 0.5 grams of methylene azure, 0.5 grams of sodium carbonate in 200 c.c. of distilled water. Cool slightly and filter. The crude methylene-violet separates out from the filtrate on cooling. Dry the crystals thoroughly.

Of the above crude methylene-violet	0.08 gr.
Of methylene-blue (med. pure)	0.08 gr.
Of eosin (water soluble yellowish)	0.20 gr.

Dissolve in 100 c.c. of methyl alcohol, filter, and dilute with 10 c.c. of methyl alcohol. The method of use is as follows:

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*MacNeal. *Journ. Infectious Diseases.* Vol. 3, 1906, pp. 412–433.

Prepare films of blood as directed in Chapter VII., Part I., and allow to dry.

(*a*) Pour the stain over the surface of the preparation till it covers it and allow to remain for one miunte. This serves to fix the film of blood to the glass as well as to stain it, so it is not necessary or desirable to pass the preparation through the flame.

(*b*) Add distilled water, drop by drop, till a reddish tint appears at the edges and a metallic scum forms on the surface.* About six drops are needed for a three-fourths inch cover-glass. The real staining of the preparation now takes place, and requires two or three minutes.

(*c*) Wash in distilled water till the thin parts of the preparation have a yellowish or pinkish tint, which requires one to three minutes.

(*d*) Dry with blotting-paper and mount in Canada balsam.

Bacteria, malarial parasities, and cell-nuclei are stained blue, red blood-corpuscles are orange-pink, while the specific granule of the leukocytes (neutrophilic, etc.) appear in varoius tints from red to dark blue. The chromatin of the malarial parasite takes a lilac to red color. The blood-plates have a bluish or purplish color and must not be confused with malarial parasites.

Hastings† gives directions of which the following are apparently the essential details: Dissolve 2 grams of sodium carbonate in 200 c.c. hot distilled water and stir in 2 grams methylene-blue (Ehrlich rectified). Bring to a boil over a fire flame or boil for 15 minutes over water bath. Replace water lost by evaporation and heat again for 10 or 15 minutes. Pour the hot solution off from the sediment and add distilled water up to 200 c.c. if necessary. Partially neutralize by the addition of 12.5 per cent. or 20 per cent. acetic acid; solution must remain alkaline. Add this to 1000 c.c. of a $\frac{1}{10}$ per

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*Leishman. *British Med. Jour.*, 1901, II., 757.

†Loc. cit.

cent. aqueous solution of water soluble yellow esoin. To this mixture add 70 c.c. or 80 c.c. of a 1 per cent. aqueous solution of methylene-blue (Ehrlich rectified). Filter. Dry the residue left on the filter paper, powder in a mortar. About .7 to 1 gram of powder is thus obtained. Dissolve in about 400 c.c. of pure methyl alcohol (Merk).

Nocht* recommends the addition of silver oxide to 1 per cent. methylene-blue solution. The silver oxide in this case is obtained by precipitating 1 gram of silver nitrate with sufficient alkali, and adding this to 100 c.c. of the 1 per cent. methylene-blue solution, and allowing the mixture to ripen for four or five days at room temperature.

Goldhorn† recommends the use of lithium carbonate instead of the sodium salt. His directions are to boil 2 grams of lithium carbonate, and 2 grams of methylene-blue in 200 c.c. of distilled water. This is done in a double boiler, and from time to time samples are examined in test-tubes to see whether the polychrome is formed as indicated by a red color. The mixture after cooling spontaneously is filtered, and the half of it is rendered distinctly acid with acetic acid. The two halves are then poured together. The rest of the procedure does not differ essentially from others described except that he recommends commercial wood alcohol in the place of pure methyl alcohol for dissolving the stain.

Wright's modification is as follows: To 100 c.c. of a 1 per cent. solution of sodium bicarbonate in water add 1 gram of methylene-blue. Place in the steam sterilizer at 100° C. for one hour. When cool add one-tenth per cent. watery solution of eosin (Grübler, yellowish, soluble in water) until the mixture loses its blue color, becomes purple, and a metallic scum forms on the surface. About 500 c.c. of the eosin solution are needed. Collect the precipitate on a filter; let it dry; make a saturated solution of the precipitate in methyl-alcohol; filter. To the quantity obtained add one-fourth as much methyl alcohol, so

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*Enzyklopaedie d. Mikros. Technik., quoted by Hastings, *Jour. Exper. Med.*, Vol. 7, 1905, p. 266. See also Duval. *Jour. Exper. Med.*, IX., 1907, p. 381.

†*Jour. Exper. Med.*, Vol. VIII., 1907, pp. 451–459.

that the solution may not be completely saturated. The purpose of the above procedures is to modify the methylene-blue so that other staining elements are developed in it (polychromism). The modified methylene-blue solution is then combined with eosin. For further details see Wright. *Journal of Medical Research.* Vol. VII., 1902.

Beside the various modifications of the Romanowsky stain given above, a number have been recommended, all of them having this in common that the nuclear stain depends upon the modification of methylene-blue due to the action of dilute alkali. Giemsa has put on the market a ready prepared stain which is essentially a modified Romanowsky. The trade name for the preparation is "Asur I. (pur.)". "Asur II" is a mixture of Asur I with equal parts of methylene-blue.

Staining of Spores.—The method is applicable to cover-glass preparations which may be prepared in the usual way from material containing spores.

(*a*) After drying the smear on the cover-glass, and fixation with heat by passing through the flame three times, use aniline-water fuchsin or carbol-fuchsin as a stain.

(*b*) Heat until the preparation begins to boil; remove for a minute; heat again, and again remove; repeat this process six times.

(*c*) Wash in dilute alcohol (Novy) or in a weak solution of acetic or hydrochloric acid for a few seconds to a minute. Some spores are quickly decolorized by 1 per cent. acetic acid; others may keep the stain when subjected to 3 per cent. hydrochloric acid alcohol for a minute.

(*d*) Wash in water.

(*e*) Stain with watery solution of methylene-blue half a minute.

(*f*) Wash.

(*g*) Dry.

(*h*) Balsam.

The spores are intensely stained by the fuchsin. The stain is removed from everything except the spores by the acid. The methylene-blue solution stains the bodies of the bacteria, the spores remaining brilliant red. There are various other methods for staining spores, but this procedure gives good results. The principle is the same as in staining the tubercle bacillus, except that more pains are needed to impregnate spores with the dye.

The spores are less readily stained than tubercle bacilli, and for this reason require prolonged and repeated treatment with the hot dye. The spores also give up the stain much more readily than tubercle bacilli, and consequently will not retain the stain if actively decolorized. Bacteria sometimes show granules of protoplasm stained by the methods used for spores, but these granules are not apt to be mistaken for spores.

Staining of Capsules.—The capsules which many bacteria possess appear to be made of gelatinous substance, which is difficult to stain.

Method of Welch.—(*a*) Cover-glass preparations are made in the usual manner. Pour glacial acetic acid over the film.

(*b*) After a few seconds, replace with aniline-water gentian-violet, without washing in water. Change the stain several times to remove all the acetic acid. Allow it to act three or four minutes.

(*c*) Wash and examine in salt solution, 0.8 to 2.0 per cent.

Bacteria are deeply stained, while their capsules are pale violet. This method has been recommended for staining the capsule of the pneumococcus.

Methods of Hiss.[*]—1. (*a*) Make cover-glass preparations in the usual manner, and fix in the flame.

(*b*) Stain for a few seconds in a half-saturated watery solution of gentian-violet.

(*c*) Wash in 0.25 per cent. solution of potassium carbonate in water.

(*d*) Mount and study in the same.

2. (*a*) Cover-glass preparations are made and fixed in the ordinary way.

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[*] *Journal of Experimental Medicine*, VI., 1905, p. 317.

(*b*) Use the following stain, heated till it steams:

Saturated alcoholic solution of gentian-violet or fuchsin	5 c.c.
Distilled water	95 c.c.

(*c*) Wash in 20 per cent. solution of cupric sulphate.

(*d*) Dry and mount in Canada balsam.

The methods of Hiss are recommended to be used for bacteria that have been cultivated on media containing blood-serum. They have shown that many streptococci have capsules. The writer has had good success from the latter method, with preparations of the pneumococcus from animal tissues.

Staining of Flagella.—Flagella are among the most difficult of all objects to stain. The best-known method is that of *Löffler.* It is important to use young cultures, preferably the cloudy water in an agar culture, or a fresh beef-broth culture.

(*a*) Spread a small portion of the culture on a cover-glass using a drop of water if necessary. The preparations must be *exceedingly thin.* The spreading must be done with care in order not to break off the delicate flagella. It is better to allow the drop of culture to run of its own accord over the cover-glass, and not stir it with the platinum needle more than is absolutely necessary. The cover-glass must be perfectly clean (see page 25).

(*b*) After drying, fixation is effected by passing through the flame three times.

(*c*) The essential point in this method is the use of a mordant as follows:

Tannic acid, 20 per cent. solution	10 c.c.
Saturated solution of ferrous sulphate	5 c.c.
Saturated alcoholic solution of fuchsin	1 c.c.

This solution is filtered and a few drops are placed on the cover-glass, or the cover-glass is placed, face down, in a dish containing the stain; it is then left for one to five minutes, warming slightly.

(*d*) Wash in water.

(*e*) Stain with aniline-water fuchsin, or carbol-fuchsin.

(*f*) Wash in water.

(*g*) Dry.

(*h*) Mount in Canada balsam.

(According to Löffler, certain bacteria require the addition of an acid solution, and certain others an alkaline solution, but many observers consider this unnecessary.)

Another and very valuable method is that of *Van Ermengem.*

(*a*) Make and fix cover-glass preparations as in the preceding method.

(*b*) Use the following mordant for one-half hour at room-temperature or for five minutes at 50° to 60° C.

Osmic acid, 2 per cent. solution	1 part.
Tannic acid, 10 to 25 per cent. solution	2 parts.

(*c*) Wash carefully in distilled water and then in alcohol.

(*d*) Place for a few seconds in a 0.25 to 0.50 per cent. solution of nitrate of silver—"the sensitizing bath."

(*e*) Without washing transfer to the "reducing and reinforcing bath:"

Gallic acid	5 grams.
Tannic acid	3 grams.
Fused potassium acetate	10 grams.
Distilled water	350 c.c.

(*f*) Ater a few seconds, replace the preparation in the nitrate of silver solution, in which it is kept constantly moving, till the solution begins to acquire a brown or black color.

Some recommend leaving the preparation in the nitrate of silver solution for two minutes in the first place, and in the reducing bath for two minutes, without using the nitrate of silver solution a second time.

(*g*) Finally wash in distilled water, dry, mount in Canada balsam. It is difficult to avoid the formation of precipitates; otherwise the results of this method are usually good.

A number of other methods or modifications of existing methods have been recommended, all giving more or less satisfactory results. The flagella are rarely or never stained uniformly throughout the preparation by any method. Those lying at the edges of the drop are most apt to be intensely stained.

CHAPTER III.

STERILIZATION.

By sterilization is meant the killing of all microörganisms found on or in any body or substance. It is possible to sterilize objects by the use of bichloride of mercury (corrosive sublimate), carbolic acid and other chemical agents, but their value in practice is often overrated. The most effective sterilization is that done with heat, either by direct application of the Bunsen burner, or by heated air, or by steam, or by boiling.

The naked flame of the Bunsen burner or the alcohol lamp is used largely for the sterilization of small articles. It is evident that no more efficient way of sterilization could be devised than by burning objects or subjecting them to a red heat. The uses of this method will at once suggest themselves; for instance, surgical dressings that have become soiled with discharges and similar materials can be most easily disposed of by simply burning them up. In laboratory work the flame is constantly employed for the sterilization of the platinum wire, forceps, pipettes and cover-glasses; occasionally test-tubes are sterilized in this manner.

Hot-air Sterilization.—Hot air, at a temperature of 150° C., or higher, maintained for an hour, is very valuable for some materials, although less effective than steam. It has been found that the spores of certain bacteria are not killed even by exposure to this temperature, but it is sufficient for ordinary conditions. Hot-air sterilization is employed for glassware, such as Petri dishes, flasks and test-tubes. Flasks and test-tubes are generally plugged with raw cotton. The heating should not be allowed to go to the extent of scorching the cotton; but a faint light browning of the outside is permissible, and is an indication that the sterilization is effectual. Glassware should be placed within the sterilizer when it is cold, and after heating should be allowed to cool gradually in order to avoid breaking. Hot-air sterilization is never used for culture-media.

The apparatus used for hot-air sterilization consists of a box made of sheet-iron, the walls being double, with an air-space between them. On one side is a door. There are openings at the top to secure the circulation of air in the air-chamber. A thermometer passes from the top into the interior of the sterilizer so that one may read off the temperature that is being attained. The sterilizer should be placed so that there will be no danger of its setting fire to inflammable articles, as the heat

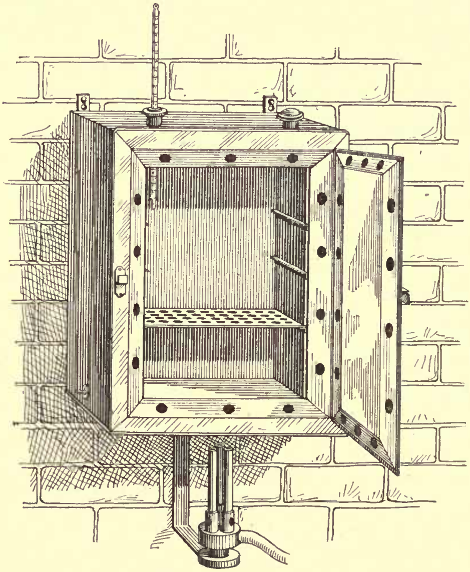

FIG. 11.—Hot-air sterilizer.

may occasionally become very intense. It is well, if possible, to have it fastened to a brick wall.

Boiling.—Boiling in water is an efficient means of sterilization for some purposes.

Steam Sterilization.—In laboratory work "live" steam or steam under pressure is generally substituted for simple boiling in the water and is more effective than the latter.

By "live" steam is meant the steam which streams off from the Koch or from the Arnold sterilizer described below. By steam under pressure is meant that generated in an autoclave also described below. But in both cases the steam to be effective must be saturated with moisture. In the Koch and in the Arnold sterilizers this is always the case; though experiments have been made in which the steam was kept at 100° C., but deprived of moisture, and in this case it was much less effective. It becomes under such circumstances merely air with more or less moisture, and experiments have shown that the sterilizing power of heated air is in proportion to the amount of moisture contained. Steam under pressure is not necessarily saturated with moisture. If water is boiled in a closed vessel the steam will be mixed with the air which is inclosed unless this is allowed to escape before the vessel is closed. This matter is of practical importance in using the autoclave.

Steam is employed for perishable bodies which would be injured by dry-air sterilization or by chemical germicides; for example, it is used for surgical instruments and for culture-media; in laboratory work, especially for culture-media. It has been found that there are some forms of bacteria which, in the resting or spore stage, can resist the action of steam even for several hours. Such prolonged exposure to steam would be injurious to culture-media, which are more or less unstable organic substances. What is called *fractional*, *intermittent* or *discontinuous* sterilization is used for such materials. By that plan the medium is sterilized with steam for fifteen minutes on each of three consecutive days. The object of intermittent sterilization, as explained by Tyndall, who proposed it, is this: The culture-medium may be supposed to contain fully developed bacteria, and also bacteria in the spore or resting stage. The first sterilization of fifteen minutes will probably be sufficient to destroy all the fully developed bacteria; during the twenty-four hours between the first and second sterilization all of the spores which have survived the first sterilization may be expected to have become fully developed into bacteria which can be destroyed by the second sterilization; the third sterilization is directed against any spore forms which may possibly have survived the second sterilization.

Although the spore forms which are so extremely resistant are non-pathogenic, as for example spores of the hay bacillus and of the potato bacillus, they nevertheless are capable of ruining the culture-media with which one works.

It has been shown by Theobald Smith that the discontinuous method cannot be relied upon to sterilize fluids in shallow layers that are freely exposed to the air. For if the spores of anaerobic bacteria happen to be present in such fluids, they will not develop into the adult form between the applications of heat, under aerobic conditions.

The sterilization of culture-media is usually effected by seven to ten minutes' sterilization in the autoclave at fifteen pounds' pressure. This is more certain than the fractional method, and may be employed without injury for all media except nutrient gelatin. The gelatinizing property of this is interfered with by the high temperature of the autoclave. It is not advised to sterilize media containing sugars in the autoclave.

The form of sterilizer widely used in the United States is that which is known as the Arnold Steam Sterilizer.

The Arnold sterilizer consists of a cylinder of tin or copper with a cover, which is enclosed in a movable, cylindrical outer cover or hood. The inner cylinder has an opening in the bottom through which steam may enter, the steam coming from a small chamber underneath with a copper bottom to which the flame is applied. The peculiarity of this form of sterilizer consists in the fact that the steam which escapes from the sterilizing chamber condenses beneath the outer cover or hood and falls back upon the pan over the chamber in which the steam is generated. The bottom of this pan is perforated with three small holes, which allow the water of condensation to return into the chamber where the steam is generated. The sterilizer, therefore, to a certain extent, supplies itself with water, although not by any means perfectly. It is, however, less likely to boil dry than other forms of sterilizers, and it has the advantage of being reasonably cheap and quite effective. The

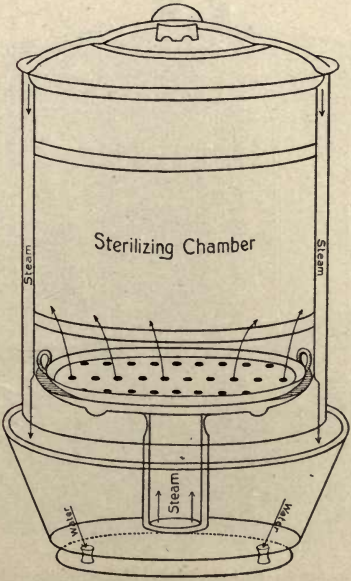

FIG. 12.—Diagram of the Arnold steam sterilizer.

space inclosed by the hood also serves as a steam-jacket and helps to overcome fluctuations in temperature. A great improvement upon the ordinary Arnold sterilizer is the modification of it devised by the Massachusetts Board of Health.

In the use of this, or any form of steam sterilizer, the time is noted from the period when boiling is brisk and it is evident that the sterilizing chamber is filled with hot steam; or, what is better, when the thermometer registers 100° C., if the sterilizer be provided with a thermometer. With a large Arnold sterilizer a temperature of 100° C. may not be reached until it has been heated with a rose-burner for twenty to thirty-five minutes. When bulky articles or large amounts of material are to be sterilized allowance must be made for the time necessary to bring the temperature in the middle of the mass to 100° C.

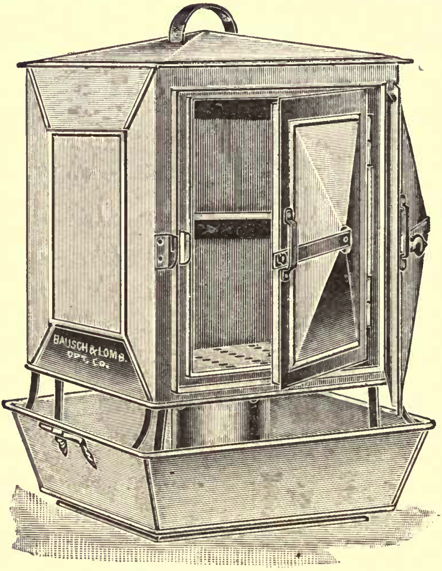

FIG. 13.—Steam sterilizer, Massachusetts Board of Health.

The sterilizer invented by Koch is still largely in use. It is a tall, cylindical, tin vessel covered with asbestos or felt. The lower portion is filled with water; on the side is a water-gauge indicating the height of the water, in order that one may observe when there is danger of the sterilizer boiling dry. Over the top there is a tight-fitting cover. The steam is generated by a Bunsen burner standing underneath. A perforated shelf placed some distance above the surface of the water is for the reception of the tubes and flasks that are to be sterilized.

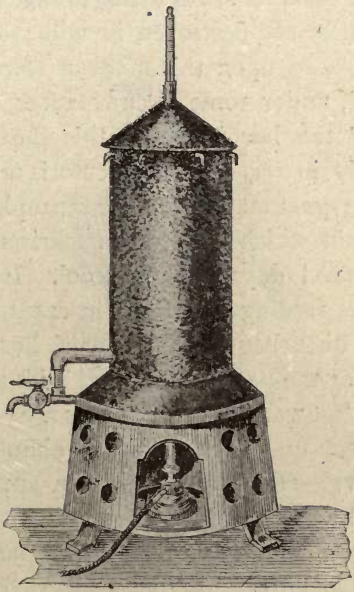

FIG. 14.—Koch's steam sterilizer.

The *sterilization* of *blood-serum* as a culture medium sometimes has to be performed in a specially devised sterilizer, when a clear, fluid medium is desired. In this case the serum is heated for an hour on each of six consecutive days to a temperature of only 58° C. To obtain a transparent but solid medium the serum is kept at a temperature of 75° C. for an hour on each of four consecutive days. The process must be conducted carefully to avoid clouding of the serum.

Pasteurization.—The name pasteurization has been applied to the partial sterilization of substances at a comparatively low temperature. It is employed particularly for milk. Although the temperature used (63° to 65° C. for 20 to 30 minutes) is sufficient to destroy all ordinary pathogenic bacteria, at least in test-tube experiments, and the probability is that where the bacteria are actually brought to the temperatures indicated they are destroyed in milk, too great reliance should not be placed upon this mode of sterilization; for, as elsewhere stated, under some circumstances milk may afford a protection for the bacteria, and this should be borne in mind particularly in regard to tuberculosis and typhoid fever. Furthermore, the great majority of the saprophytic bacteria are destroyed, and milk which has been pasteurized will remain unchanged for several days, if kept cool. Its application is principally in the feeding of infants in cases where raw milk causes digestive disturbances. Freeman* has invented a pail of special form for the pasteurization of milk in bottles. This pail is filled with hot water and the bottles are placed in it; it has been found to keep up a temperature of about 75° C.

The Autoclave.—The autoclave is an apparatus designed for sterilization by steam under pressure. It was invented in France, but is now used extensively in all parts of the world. Steam generated at the ordinary atmospheric pressure is much less destructive to bacteria, and especially to their spores, than steam in the autoclave at a pressure of an additional one-half to one atmosphere, 7½ to 15 pounds; the steam then reaches a temperature of about 112° to 120° C. Under these conditions culture-media may be sufficiently sterilized in the autoclave in ten minutes, and at a single sterilization. The autoclave consists of a metal cylinder with a movable top, which is fastened down tightly during sterilization. It is furnished with a thermometer, a pressure gauge, a stop-cock, and a safety-valve which is set to allow the steam to escape when the desired pressure is attained and thus prevents it from running too high. Heat is furnished by a gas-burner underneath. The lower part of the cylinder contains water. The objects to be sterilized are supported above this water on a perforated bottom or shelf.

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*Medical Record. July 2, 1892, and August 4, 1894. This pail is sold by James T. Dougherty, 411 West Fifty-ninth Street, New York.

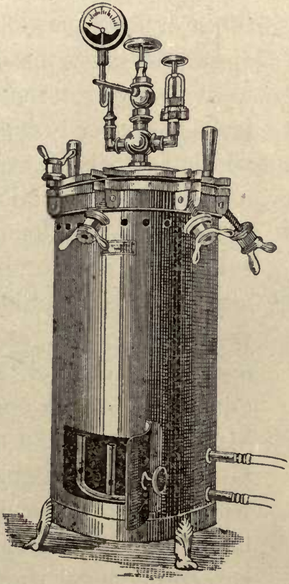

FIG. 15.—Autoclave.

It is necessary to follow certain precautions in the use of the autoclave. Allusion has already been made to the necessity for having the steam saturated with moisture. This is effected by allowing the air to escape after the heat is applied, and in order to be sure that all the air has really been expelled, the stop-cock, with which all autoclaves are provided, is left open until the steam escapes freely. The stop-cock is then closed, and the pressure begins to rise. After leaving the articles to be sterilized in the autoclave for the length of time desired, the apparatus must not be opened while the steam contained within it is still under pressure, as there may be a sudden evolution of steam upon the removal of the pressure which may blow the media out of their tubes and flasks. On the other hand, the pressure must not be allowed to drop below zero, for in this case the plugs of the tubes or flasks may be sucked in. The apparatus must, therefore, be kept closed until the gauge shows that the atmospheric pressure is as great as the pressure within, or, what is equivalent, until the temperature has fallen to 100° C. but not below this temperature. A good rule is to watch until the pressure is very near zero, and the temperature very near 100° C., and then to cautiously open the stop-cock a very little. The autoclave may be opened as soon as the hissing from the out-driven steam ceases. Gelatin may be damaged by sterilization with the autoclave, if it be heated too long or too high a temperature. Media containing sugar should not be sterilized in the autoclave (see page 67).

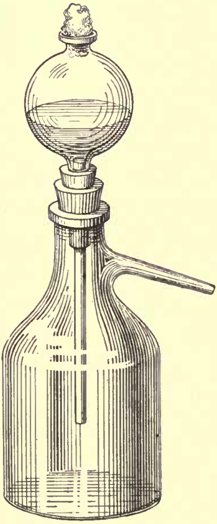

FIG. 16.—Kitasato filter.

Sterilization by Filtration.—Ordinary filters are useless for this purpose, but the tubes or bougies of unglazed porcelain devised by Pasteur and Chamberland are effective when properly employed. They are made in several different grades of porosity. In the Berkefeld filter bougies made of infusorial earth are used, and the pores in this are larger than those of the Pasteur filter. The coarser of these filters permits the passage of very small bacteria. Bacteria of average size, like bacillus coli communis, may grow through the pores in the walls of both the Berkefeld and Pasteur filters if sufficient nutrient material is present to permit of their multiplication.*

Filters of these kinds are widely used for water, and will be spoken of in connection with the chapter on water. Similar tubes are employed for the filtration of certain organic nutrient media whose ingredients would be damaged by sterilization with heat, chiefly extracts of organs. The soluble "toxins" of bacteria may be obtained by filtration of fluid-cultures through such tubes, which remove the bacteria (Fig. 16). These fluids usually filter very slowly, and filtration has to be assisted by some form of vacuum-pump; usually the filter-pump, which is used in connection with a stream of running water, is employed. Compressed air or carbonic acid may be used to assist in forcing fluids through the filter. The filter bougies, the flasks and all parts of the apparatus must, of course, be sterilized by heat before and after using.

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*Wherry. *Journal of Medical Research.* Vol. VIII., 1902.

CHAPTER IV.

CULTURE-MEDIA.

CULTURE-MEDIA are substances in which bacteria are artificially cultivated. The number of such substances is very large, different materials being suited to different purposes and to different kinds of bacteria. The most important ones are nutrient bouillon or beef-tea, nutrient gelatin, and nutrient agar-agar. The two last have a jelly-like consistency, owing to the addition of a gelatinizing substance, but otherwise are of the same composition as the bouillon.

In all cases the media must be either free from bacteria originally or they must be rendered free from bacteria in order that the organisms under cultivation may be studied in pure cultures. This is effected, as a rule, by steaming in the steam sterilizer or in the autoclave. For special purposes filtration through porcelain filters is resorted to.

Preparation of Media.

NUTRIENT BOUILLON.

Beef-extract (such as Liebig's)	3 grams.
Peptone, pure (Witte's)*	10 grams.
Sodium chloride (common salt)	5 grams.
Water	1 liter.

The solid ingredients are dissolved in water, and the mixture is boiled for a few minutes. It is made neutral or very faintly alkaline by the addition of a solution of sodium hydroxide, drop by drop, the reaction being tested at intervals with litmus-paper. As soon as the proper reaction is reached, it is filtered through filter-paper. The filter-paper should be folded and creased as is done by pharmacists; it is in the usual manner placed in a glass funnel, and should be moistened with water before using. After filtration the medium is to be placed in properly plugged tubes or flasks, and is to be sterilized once in the autoclave, or in the steam sterilizer for fifteen minutes or longer on each of three consecutive days. When precipitates form, they are usually caused by a too alkaline reaction. That may be corrected by the addition of a little weak hydrochloric acid, drop by drop, testing frequently with litmus-paper.

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*Commercial "peptones" are mixtures of albumose and a small amount of peptone.

A more accurate way of obtaining the proper reaction is Schultz's method. Take of the bouillon 10 c.c.; add a few drops of phenolphthalein* (alcoholic solution, ½ per cent.); with a burette add, drop by drop, a solution of caustic soda, 0.4 per cent., until a faint red color appears, which indicates the beginning of the alkaline reaction. This procedure is followed with three samples. The amount of soda solution required in each case is noted and the average taken. If now, on the average, for each 10 c.c. of bouillon 1 c.c. of soda solution needs to be added, for 1000 c.c. of bouillon 100 c.c. of the soda solution must be added; only, instead of adding a weak soda solution, one-tenth as much is taken of a solution ten times as strong.

Another method of making bouillon and that most usually recommended, is to use, instead of beef-extract, 500 grams (one pound) of finely chopped, lean beef, which is placed in one liter of water and kept on ice for twenty-four hours. It is strained, thoroughly cooked to coagulate the albumen in it, filtered and a liter of fluid obtained, adding water if necessary. The peptone and salt are then added and the medium heated to dissolve them. Some advise the addition of the peptone and salt to the fresh watery extract of the meat before boiling. In preparing media for the purpose of water analysis it is advised to leave out the salt. It is then neutralized, filtered and sterilized. Although bouillon made with solid beef-extract is con-

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*In neutralizing an acid culture-medium it has been found that when the medium appears to be neutral or slightly alkaline to litmus, it may still be acid if phenolphthalein be employed as an indicator. Fuller. *Journal American Public Health Association.* 1895.

venient and serviceable for most purposes, it is advisable to use meat when the bouillon is to be employed for the development of bacterial toxins. Meat should also be used in the preparation of either bouillon, gelatin or agar-agar when new species of bacteria are being studied for publication.

In both of these cases the recommendations of the American Public Health Association should be followed.*

These recommendations have been largely followed in the directions for the preparation of culture-media given below. The student is referred to the report itself for further details than those given below.

The following solutions are required: ½ per cent. phenolphthalein in 50 per cent. alcohol, normal† $\left(\frac{N}{1}\right)$ and twentieth normal $\left(\frac{N}{20}\right)$ solutions of sodium hydroxide and of hydrochloric acid.

To 5 c.c. of bouillon in a porcelain evaporating dish add 45 c.c. of distilled water; boil three minutes; add 1 c.c. of phenolphthalein solution, and proceed with the titration while still hot. As the reaction will usually be found acid, add from a burette $\frac{N}{20}$ sodium hydroxide solution, stirring constantly, until a decided pink color develops in the entire solution. The color reaction indi-

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*Report of Committee of the Am. Pub. Health Assn. on Standard Methods of Water Analysis, *Journ. Infect. Dis.* Sup. No. 1. May, 1905.

†A normal solution of any substance contains, in a liter, as many grams of the substance as there are units in its molecular weight, in case it contains a single atom of replaceable hydrogen. If it has two atoms of replaceable hydrogen the number of grams used equals the molecular weight divided by two; and so on. Thus the molecular weight of sodium hydroxide is 40, and its normal solution contains 40 grams of sodium hydroxide in a liter. It is not expedient to prepare normal solutions of sodium hydroxide by weight. For convenience, crystallized oxalic acid is used as a starting point in making normal solutions. Its molecular weight, including a molecule of water of crystallization, is 126. As it is a dibasic acid (having two atoms of replaceable hydrogen), half of this weight, or 63 grams, per liter, is taken. Any $\frac{N}{1}$ acid solution will exactly neutralize an equal volume of any $\frac{N}{1}$ alkaline solution. To make $\frac{N}{1}$ sodium hydroxide solution, add about 41 grams of pure caustic soda to a liter of distilled water. Find the amount of this solution needed to exactly neutralize 1 c.c. of $\frac{N}{1}$ solution of oxalic acid; this amount contains the quantity of sodium hydroxide which should be present in 1 c.c. of a normal solution. It is now possible to calculate the amount of distilled water to be added in order that 1 c.c. of the sodium hydroxide solution may neutralize 1 c.c. of the $\frac{N}{1}$ solution of oxalic acid. With an $\frac{N}{1}$ solution of sodium hydroxide as a standard, an $\frac{N}{1}$ solution of hydrochloric acid may be prepared. Twentieth normal solutions have one-twentieth the strength of normal solutions.

cates the more or less arbitrarily adopted neutral point. Repeat this procedure with three different portions of bouillon, and determine the average amount of $\frac{N}{20}$ sodium hydroxide required. It is now possible to calculate the amount of $\frac{N}{1}$ sodium hydroxide needed to neutralize the whole quantity of bouillon. This should be added. The bouillon should then be boiled for ten minutes, and again titrated. It will usually be found acid. The deficiency should be corrected by adding the necessary amount of $\frac{N}{1}$ sodium hydroxide. It should be boiled again, and again titrated, and any deficiency made good. It is rarely necessary to repeat the process, except to determine that the neutral point has been reached. After neutralizing it is boiled thirty minutes and filtered. Enough $\frac{N}{1}$ hydrochloric acid or sodium hydroxide is added to give the degree of acidity or alkalinity desired. It is then sterilized.

An acid reaction may be denoted by +, an alkaline by —. The degree of acidity or alkalinity may be indicated by the amount of $\frac{N}{1}$ solution required to render the medium neutral to phenolphthalein, thus + 1.00 signifies that a medium is acid, and requires 1.00 per cent. of $\frac{N}{1}$ sodium hydroxide to neutralize it.

A reaction of + 1.00 is recommended as the optimum. There is much disagreement as to what reaction is most favorable for the growth of the majority of species of bacteria. Even + 0.5 may be better for some bacteria. In any case the degree of reaction should be noted in descriptions.

Bouillon may be modified by the addition to it of other substances, the most important of which are glycerin (6 per cent.) and sugars,—as dextrose,* saccharose or lactose (1 per cent.). It is better to sterilize media containing sugars in the steam sterilizer by the fractional method rather than in the autoclave, for the reason that at the high temperature of autoclave decomposition of the sugars may occur.

Sugar-free Bouillon.—Ordinary bouillon often contains some muscle-sugar, which is objectionable if fermentation tests with lactose or saccharose are to be made. Muscle-sugar must also be removed from the beef-juice in growing diphtheria cultures for the production of antitoxine. To secure bouillon free of sugar, the water is added to the finely chopped beef as in other cases, but it is then inoculated at once before any further preparation with a culture of B. coli communis and allowed to stand in the incubator for twelve or fifteen hours. Early next morning it is boiled, filtered, peptone and salt added, and the bouillon is prepared as usual.† The medium should be tested for the presence of indol before it is used for diagnostic purposes; since Rivas* finds that in fermenting meat-juice with B. coli by Smith's method for sugar-free broth, indol may be formed.

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*Dextrose is the principal ingredient of commercial grape-sugar or glucose and should be obtained in a pure condition.

†See Theobald Smith. *Journal of Experimental Medicine*. Vol. II., 1901, p. 546.

Beef-extract	3 grams.
Pepton	10 grams.
Sodium chloride	5 grams.
Gelatin (best gold label)	100 grams.
Water	1 liter.

Dissolve the ingredients in the water, stirring actively to prevent burning at the bottom. It is best to conduct the operations in granite or enamel-ware vessels over a large Bunsen or rose-burner. Neutralize with sodium hydroxide solution (see page 66). Litmus-paper or titration may be used for testing. The reaction at the beginning will usually be found to be quite acid. Allow the mixture to cool until below 60° C., and add the whites of one or two eggs which have been beaten up with a little water; stir in thoroughly. Heat the mixture to the boiling-point; stir at the bottom to prevent burning and at the same time avoid as far as possible breaking the coagulum of egg-albumen which forms at the surface. Boil for ten minutes. Filter while hot. The filtration may be done through folded filter-paper which has been moistened. It is well to fasten a piece of coarse cheese-cloth over the top of the funnel to catch the large particles of coagulated albumen. Place in suitable tubes or flasks plugged with cotton, and sterilize once in the autoclave, or, preferably, in the steam sterilizer for fifteen minutes on each of three consecutive days. Gelatin is injured by too prolonged boiling and loses its solidifying qualities. The remarks on pages 66 to 67 with regard to the use of beef and the titration method for the preparation of bouillon apply equally to gelatin.

Instead of filter-paper, some prefer to filter through several layers of absorbent cotton placed inside of the moistened glass funnel, the top of which is covered with coarse cheese-cloth.

***Journal of Infectious Diseases.* Vol. IV., No. 4, Nov. 15, 1907, pp. 641–646.

This expedient answers very well, but filtration through paper is apt to give better results.

If the product appears cloudy after it has been sterilized, it may be that the egg-albumen was incompletely coagulated in the first place or that the reaction has been made too alkaline. In any case it will be desirable to melt it and filter a second time, correcting the reaction with hydrochloric acid if necessary. It may be well to stir in another egg to entangle the opaque particles; then to boil a second time and filter.

The medium is sometimes modified by adding to it other substances, as sugar, glycerin, etc. The solidifying property of the gelatin must be carefully guarded, and too much boiling is to be avoided. Certain bacteria, it will be found, have the property of causing gelatin to become permanently liquid: this is called *liquefaction* or *peptonization.* Gelatin melts at about 25° C. and solidifies at about 10° C. It cannot be used in the incubator, where it would melt at the temperature of 38° C. In hot weather it may be necessary to use 150 grams of dry gelatin to the liter. Nutrient gelatin is usually spoken of simply as "gelatin."

Nutrient Agar-agar.—Agar-agar (French, *gélose*) is a kind of vegetable gelatin which comes from the southern and eastern coast of Asia. It melts with much greater difficulty than gelatin, and remains solid at much higher temperatures. In this respect it behaves very peculiarly, since it will not melt unless it is heated to about 80° C.; but after it is once melted it remains fluid at 40° C., or over. After it solidifies it has to be heated up to about 80° C. before it will melt again.

The medium is not quite transparent. The finished medium is commonly called "agar."

Beef-extract	3 grams.
Peptone	10 grams.
Sodium chloride	5 grams.
Dry Agar	15 grams.
Water	1 liter.

The dry agar, cut fine, is to be dissolved in water over a flame or in the autoclave. It should be boiled for from one-half hour to two hours, skimming off the scum which forms on the surface from time to time. The beef-extract, peptone and sodium chloride are dissolved in a liter of water, boiled and neutralized. Add the agar now in solution in a small quantity of water. The reaction of the agar alone is faintly alkaline. Mix thoroughly; the bulk of the mixture is a little more than a liter, and should be reduced to a liter by the subsequent boiling. Cool to about 60° C.; stir in the whites of one or two eggs and boil thoroughly. Avoid breaking the coagulum of egg which is designed to entangle the solid particles that make the medium cloudy; stir at the bottom, however, to prevent burning. Filter while hot, using filter-paper or absorbent cotton covered with cheese-cloth. The hot-water funnel originally devised for the filtration of agar is not necessary. If filtration is slow, the funnel and flask may be placed inside of the steam sterilizer and kept heated during filtration. The medium is collected in suitable flasks or tubes plugged with cotton, and sterilized once in the autoclave or in the ordinary steam sterilizer for fifteen minutes on each of three consecutive days. As agar is frequently used for smear-cultures where a slanted medium is desired, some of the tubes may be allowed to cool in a slanting position. It is not well to keep on hand many tubes which have been slanted, as the medium dries more rapidly. Agar is seldom liquefied by bacteria, though a few bacteria possess the power of doing this. Its solidifying qualities are impaired somewhat if the reaction be acid.

The remarks on pages 66 to 67 with regard to the use of beef and the titration method for the preparation of bouillon apply equally to agar-agar.

Glycerin-agar is used extensively. It is agar, made as above directed, to which 6 per cent. of glycerin is added before

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sterilization. It is very useful in cultivating the bacilli of tuberculosis and diphtheria.

Sugar-agar.—Before sterilizing, 1 per cent. of either dextrose, lactose, saccharose or other sugars may be added to agar. In this case the agar should be prepared from sugar-free broth as in the preparation of broth to which the sugar, are added. With media containing sugar, *litmus* forms a useful indicator of the production of acid. Enough tincture of litmus is used to give the medium a blue color before sterilization; the litmus is somewhat unstable and prone to change its color during sterilization. *Azolitmin* is now recommended in place of litmus (see below). *Neutral red* may also be added in the same manner; its color is changed by certain bacteria and not by others (see bacillus of typhoid fever and bacillus coli communis, Part IV.). To 1 liter of nutrient agar, add 1 gram of dextrose and 0.05 gram or 10 c.c. of a saturated aqueous solution of neutral red. Sterilize as usual.

The committee on Standard Methods of Water Analysis, Am. Pub. Health Assn.* recommend the following: Lean meat should be used, not beef extract, as a basis for the various media; sodium chloride shall not be used for water analysis; Witte's peptone shall be used; gelatin must be the best French brand, and the 10 per cent. solution after its preparation as a culture medium shall not soften at 25° C. All sugars shall be chemically pure, glycerin when used must be double distilled. In place of litmus, where this is used, azolitmin shall be substituted.

Potato.—The potatoes are washed, a slice is removed from each end, and with an apple-corer or cork-borer a cylinder is cut out.† This cylinder is divided diagonally into two pieces.

Jour. Inf. Diseaes, Supplement No. 1, May, 1905, p. 104.

†Bolton. *The Medical News*. Vol. I., 1887, p. 318. A Method of Preparing Potatoes for Bacterial Cultures. Roux. De la Culture sur Pomme de Terre. *Annales de l'Institut Pasteur*. T. II., 1888, p. 28. Globig. Ueber einen Kartoffel-Bacillus mit ungewöhnlich widerstandsfähigen Sporen. *Zeitschrift für Hygiene*. p. 322, 1888.

The pieces are washed in running water for several hours. They are placed in test-tubes containing a little water to keep the potato moist, and are supported from the bottom on a piece of glass tubing about 1 to 2 cm. in length (or on cotton, or in a specially devised form of tube with a constriction at the bottom). The tubes are plugged, and sterilized as with other media. Sterilization, however, must be thorough on account of the danger of contamination with the extremely resistant spores of the potato bacillus. Potato is best when freshly prepared; it is likely to become dry and discolored with keeping. It is a very useful medium; certain growths on it, like those of the bacillus of typhoid fever or of glanders, and those of chromogenic bacteria, are very characteristic.

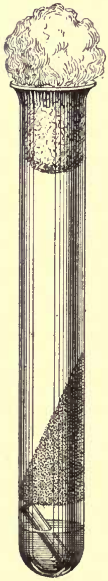

FIG. 17.—Tube containing potato.

Milk.—Milk fresh as possible is placed in a covered jar, sterilized for fifteen minutes, and then kept on ice for twenty-four hours. At the end of that time the middle portion is removed by means of a siphon. The upper and lower layers must not be taken; the upper part contains cream, and the lower part particles of dirt, both of which are to be avoided.

The reaction should be corrected to + 1 if the milk is found to be too acid. About 7 to 10 c.c. are to be run into each test-tube. The tube is plugged with cotton and sterilized as usual. When milk is contaminated with spores of the hay or potato bacillus it is sometimes very difficult to sterilize, a fact of much importance in connection with the feeding of children, where the fractional method of sterilization and the use of the autoclave are impracticable.

The coagulation of milk, which is brought about by certain bacteria, is a very valuable differential point. Litmus milk is prepared as above and has added to it 1 per cent. of azolitmin before sterilizing. This indicates whether or not acids are formed by the bacteria which are afterward cultivated in the milk.

DUNHAM'S PEPTONE SOLUTION.

Peptone	10 grams.
Sodium chloride	5 grams.
Water	1 liter.

Boil, filter, sterilize in the usual manner.

Nitrate Broth.—Dissolve 1 gram of peptone in 1000 c.c. of tap water, and add 2 grams of nitrite-free potassium nitrate. This solution is distributed into test-tube, 10 c.c. in each tube.

Broth for the Indol Test.—Standard broth described above may be used for this test provided it contains no muscle-sugar, the muscle-sugar having been removed by cultivating B. coli in the beef infusion for twenty-four hours previous to its preparation. Or the following solution may be employed for the test:

Dunham's solution is valuable to test the development of indol by bacteria (see Part II., Chapter II.). The development of acids may be detected after the addition of 2 per cent. of rosolic acid solution (0.5 per cent. solution in alcohol); alakaline solutions give a clear rose-color which disappears in the presence of acids.

Blood-serum.—The blood of the ox or cow may be obtained easily at the abattoir. It should be collected in a clean jar. When it has coagulated, the clot should be separated from the side of the jar with a glass rod. It may be left on the ice for from twenty-four to forty-eight hours. At the end of that time the serum will have separated from the clot and may be drawn off with a siphon or pipette into tubes. The tubes containing the serum should be placed in a slanting position, as nearly horizontal as possible without bringing the serum in contact with the cotton plug, and while in this position they should be heated gradually up to 65–68° C. This is best done in a specially constructed Koch serum coagulator, but it may also be done as advised by Councilman and Mallory, in the hot-air sterilizer at a temperature below the boiling-point or it may be accomplished by means of a water bath or in the Arnold sterilizer or in other ways. The more gradually the heat is raised and the lower the temperature at which the serum is coagulated the more transparent it remains. After coagulation the tubes of serum may be sterilized in the Arnold sterilizer on three successive days, or they may be sterilized in the autoclave at 110° at one time. Sterilization of blood-serum in the autoclave is not recommended by some authors.* Blood-serum may be sterilized in the special form of sterilizer devised for it. A clear fluid blood-serum may be obtained by sterilization at a temperature of 56 to 58° C. for one hour, on each of six days. Opaque, coagulated blood-serum has most of the advantages of the clear medium. Blood-serum may be secured from small animals by collecting blood directly from the vessels, using very great care to obtain the blood in a sterile condition; and the serum may be separated and stored in a fluid state. Human blood-serum is sometimes obtained from the placental blood, sometimes from serous pleural transudates or from hydrocele fluid. The preservation of blood-serum is sometimes accomplished with chloroform, of which 1 per cent. is to be added to the medium; in this manner the serum may be preserved for a long time. It may be divided into tubes, solidified and sterilized as required; the chloroform is driven off by the heat, in sterilizing, but it must be heated to at least 68° C. Blood-serum media which are sterilized at low temperatures should be tested for twenty-four hours in the incubator to prove that sterilization has been effective; if it has not, development of the contaminating bacteria will take place and be visible to the eye.

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*Park. Pathogenic Bacteria and Protozoa. New York and Phila., 1905. p. 51.

It will be impossible to do more than merely mention some of the most important of the other culture-media.

Loffler's blood-serum consists of one part of bouillon containing 1 per cent. of glucose and three parts of blood-serum. It is sterilized like ordinary blood-serum. It is used largely for the cultivation of the bacillus of diphtheria.

Blood-serum-agar is a medium made with considerable difficulty, but when made with human blood-serum very valuable for the cultivation of the gonococcus. One part of placental blood-serum, or pleuritic serum, or hydrocele fluid, is mixed with one to two parts of nutrient agar in the fluid condition. It must be divided into tubes before solidification. It should be solidified in a slanting position, and sterilized at 58° C. so as not to coagulate the blood-serum. At this temperature it is necessary to sterilize for several successive days (see page 74) and it should be tested in the incubator for sterility. The nutrient agar in this case should contain 2 per cent. of dry agar.

Another expedient has also been to smear a little blood, drawn from a puncture made by a sterile needle in the carefully cleaned finger, over the surface of a tube of nutrient agar—*blood-agar*—used for cultivating the bacillus of influenza. In this case the finger from which the blood is drawn is scrubbed with soap and water, soaked with $\frac{1}{1000}$ corrosive sublimate, and finallywashed with alcohol. Small quantities of blood may be drawn from a vein in the ear of a rabbit (see page 103) with a sterile hypcdermic syrince, and is quickly divided among three or four tubes of agar, melted in the upper third; slant the tubes while cooling. To make a large amount of blood-agar, bleed a rabbit from the carotid artery into a sterile flask containing pieces of sterile glass tubing; shake the flask constantly; divide the defibrinated blood among tubes containing sterile nutrient agar; slant the tubes while cooling. Use one part of blood to about two of agar. Great care must be used not to contaminate the blood as it is drawn, and it is best to test it in the incubator for sterility. The tubes when completed should stand some days before using, so that contaminating bacteria if present may grow in the interval and permit such tubes to be discarded.

Guarnieri's medium consists of a mixture of gelatin and agar.

Eggs in their shells may be used after sterilization by steam, which of course coagulates the albumen. The egg is easily inoculated through a small opening made with a heated needle, which may be closed afterward with collodion. Egg-albumen has been used as a constituent of various media. Dorset* has found that eggs furnish an excellent culture-medium for tubercle bacilli. The yolk and the white are mixed, poured into tubes, slanted, coagulated, and sterilized. Just before using pour into the tube a few drops of sterile distilled water to moisten the medium. This is a most valuable addition to the technic.

Bread-paste (finely-divided dry bread, mixed with water and sterilized) is used for the cultivation of moulds. *Sabouraud* recommends the following for the cultivation of the trichophyton fungus:

Peptone	5.0 grams.
Maltose	3.8 grams.
Agar	1.3 grams.
Water	100.0 c.c.

Test-tubes.—Bacteria are generally cultivated in test-tubes. A convenient size is one $\frac{5}{8}$ of an inch in diameter and 5 inches in length. The tubes should be of a heavier glass than in those used for ordinary chemical work. The New York Board of Health, and some others, use a tube three inches in length without a flange for the cultivation of the diphtheria bacillus on Löffler's blood-serum mixture. Test-tubes should be thoroughly cleaned with a swab before using; they should be boiled with washing-soda, rinsed, filled with hydrochloric acid solution, rinsed and inverted to drain away the fluid.

Plugs of raw cotton or cotton batting are employed as stoppers. Some prefer absorbent cotton, but it is likely to become soggy after exposure to steam. The plug should fit smoothly; creases and cracks around the edges are to be avoided. The plug should be tight enough to sustain the weight of the tube when held by the plug. These plugs prevent bacteria from entering or leaving the tubes.

Sterilization of Test-tubes.—The tubes are to be sterilized in a hot-air sterilizer for one hour, at a temperature of 150° C. They may be left in until the cotton acquires a light-brown color, but it should not be burned. If the plugs touch the sides of the sterilizer or lie against the bottom they may be scorched.

**American Medicine.* April 5, 1902.

The necessity for sterilization of the tubes before filling them with the medium has been questioned, and it is probably unnecessary as far as the preservation of the culture-medium is concerned, but it will be found that the cotton plugs fit much better after sterilization with dry heat. During this and subsequent sterilizations the tubes are held in a wire basket.

Filling of the Tubes.—A special funnel closed with a stopcock for filling tubes with liquefied media is often recommended. They may readily be filled with an ordinary funnel of small size. During the filling, the neck of the test-tube where it comes in contact with the cotton *must not be wet* with the medium. Ordinarily about 7 to 10 c.c. are placed in a test-tube. For Esmarch's roll-tubes a somewhat smaller quantity is desirable.

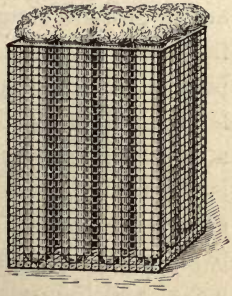

FIG. 18.—Wire basket for test-tubes.

The sterilization of tubes containing culture-media is always done by steam and has been sufficiently described. It is to be remembered that the solidifying power of gelatin is impaired by too prolonged heating, while heating is less likely to damage other culture-media. The media which are sterilized at a low temperature (70° C). should be tested for two days in the incubator to determine whether sterilization has been effective. It is the universal experience in bacteriological laboratories that occasionally culture-media will become contaminated with extremely resistant spores which fail to be sterilized by the ordinary processes, an occurrence which causes great annoyance and calls for the exercise of much patience. Sometimes, also, moulds attach themselves to the plugs, especially if they are moist, and send their filaments down through the cotton; finally, having reached the lower edge of the cotton, their spores may fall upon the medium, grow there and ruin it.

CHAPTER IV.

THE CULTIVATION OF BACTERIA.

Inoculation of the Tubes.—The air of the laboratory should be as quiet as possible, in order to lessen the chances of contamination by bacteria clinging to particles of dust. Spores are blown from the surfaces of moulds like thistle-down, and are constantly being wafted about in the air where there are draughts. After the colonies are obtained on the plate or in the Esmarch tube a pure culture is obtained by transferring a minute amount of the growth from a colony over into a test-tube containing the sterile culture medium. The transfer is effected by means of a straight platinum wire, or with a platinum wire loop. The platinum is to be sterilized by heating to a red heat in the Bunsen flame or with an alcohol lamp before using, and then allowed to cool. It is also to be heated red-hot after using. The plug of the test-tube is withdrawn, twisting is slightly, taking it between the third and fourth fingers of the left hand, with the part that projects into the tube pointing toward the back of the hand. It must not be allowed to touch any object while out of the tube. The upper inch or two of the tube should be passed through the flame in order to destroy any bacteria which may settle on it from the air while the plug is out. If any of the cotton adheres to the inside of the tube it should be removed with sterilized forceps, while the neck of the tube touches the flame, so that the threads of cotton may be burned and not fly into the air of the room. The tube should be held as nearly horizontal as possible. The tube should be held in the left hand between the thumb and forefinger, resting upon the palm, with the mouth pointing upward and to the right. When two tubes are being used at the same time, as is often necessary, they are placed side by side between the thumb and forefinger of the left hand. The two plugs are held between the second and third and the third and fourth fingers of the left hand, respectively. Inoculation from one tube to the other is effected by touching the tip of the platinum wire to the material to be transferred in the one tube, and introducing it into the culture-medium contained in the other tube. When the needle is introduced into or removed from either tube it should not touch the side of the tube at any point, but be brought in contact only with the medium to be inoculated. After inoculation of the tube has been effected the wire must be heated to a red heat in the flame, the mouths of the tubes passed through the flame, and the plugs returned to their respective tubes. When the wire to be sterilized is wet it should be approached to the flame gradually, so as to dry the material on it, in order to avoid "sputtering" (see page 22). It is well from the start to train one's self to *sterilize the platinum wire every time it is taken from the table and before it is laid down again.* The platinum loop is used in place of the straight wire when it is desired to transfer larger amounts of material than can be done at one time on a straight wire,

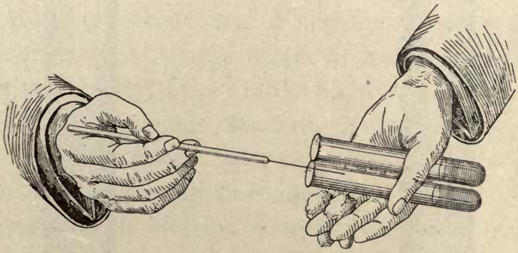

FIG. 19.—Manner of holding tubes.

and is employed in case of material containing a small number of bacteria.

When a tube of gelatin is to be inoculated the wire is usually introduced into the medium vertically, "stab-culture;" when a medium with a slanted surface is employed, as agar, potato or blood-serum, the needle should lightly streak the surface, "smear culture" (Figs. 20 and 21).

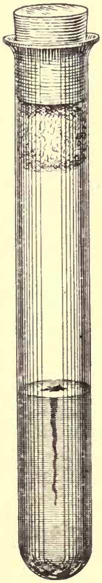

FIG. 20.—Stab culture. A rubber stopper may be use to prevent drying, see page 86.

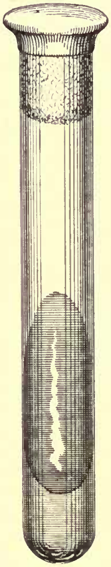

FIG. 21.—Smear culture. This tube shows the rubber cap used to prevent drying.

The safety and success of this method of inoculation depend upon a principle which has been established by long and repeated observation,—namely, that bacteria do not of themselves leave a moist surface, although they may be readily shaken off as would appear from Flügge's* results. Nevertheless, they will not rise from the surface of the moist culture-medium, nor drop from the needle during its transit, if proper care be exercised. They may be thrown into the air if the needle be allowed to sputter in the flame, or is roughly shaken about.

**Zeitschrift. f. Hygiene Bd.* 25, 1897, p. 179.

It must be remembered also that such organisms as moulds develop spores which are formed on filaments elevated above the surface of the medium and are easily detached.

If, by any accident, drops of infectious material should fall upon a surface like the table, they should be covered at once with bichloride of mercury solution 1–1000. A good way is to cover the spot with a piece of blotting-paper wet with the solution, place a bell-jar over it and leave for several hours. If infectious material should reach the hands or clothing, they should be thoroughly soaked in the bichloride solution. When working with pathogenic bacteria it is well to wash the hands in this solution and with soap and water, as a routine procedure, before leaving the laboratory.

To maintain their vitality bacteria need to be transplanted from time to time from the medium in which they are cultivated to fresh medium; the frequency with which this transplantation is necessary varies greatly with different species. *When transplanted from one tube to another the bacteria should be examined with the microscope both in the original tube and in the resulting growth in order to check the purity of the culture.* Bacteria differ greatly in the ease with which they may be cultivated artificially. Many of them grow on culture-media with difficulty when first taken from the animal body, or when first transplanted from one sort of medium to a different one. But they become accustomed to such changes of conditions and frequently may be propagated easily on the new medium. This is especially true of bacillus tuberculosis.

From what has just been said, it is evident that some bacteria flourish better on one culture-medium than on another. The bacillus tuberculosis grows best on boiled egg, blood-serum and glycerin-agar; the bacillus of diphtheria grows best on Löfflers blood-serum; the gonococcus on human serum-agar.

The virulence of most pathogenic bacteria becomes diminished after prolonged cultivation upon artificial media. Sometimes the virulence is lost very quickly,—for example, the streptococcus pyogenes and micrococcus lanceolatus of pneumonia. These organisms are often very strongly pathogenic for experiment animals, when inoculated directly from one animal to another, but frequently lose all pathogenic power, even on one transfer to artificial media.

Incubators.

High-temperature Incubator—Many bacteria flourish best at a temperature about that of the human body, 38° C. Some species will grow only at this temperature. The pathogenic bacteria in particular, for the most part, thrive best at a point near the body temperature, and are consequently best studied for many purposes when grown in an incubator, regulated for this temperature.

The high-temperature incubator used in laboratories consists essentially of a box made of copper, having double walls, the space between the two being filled with water. The outer surface is covered with some non-conductor of heat, such as felt, asbestos or linoleum. At one side is a door, which is also double. The inner door is of glass, the outer door is of copper covered with asbestos. At one side is a gauge which indicates the level at which the water stands in the water-jacket. The roof is perforated with several holes, some of which permit the circulation of the air in the air-chamber inside the box; some of them enter the water-jacket. A thermometer passes through one of these holes into the interior of the air-chamber, and often another into the water standing in the water-jacket. A gas-regulator passes through another hole, and is immersed in the water standing in the water-jacket. There are various forms of gas-regulators more or less complicated. In general they consist of a tube containing mercury; into this tube are two openings, one for the entrance and the other for the exit of gas. The gas enters through a small tube, which is cut off diagonally at the bottom, and which projects into the surface of the mercury. Heating the water in the water-jacket causes expansion of the mercury, which

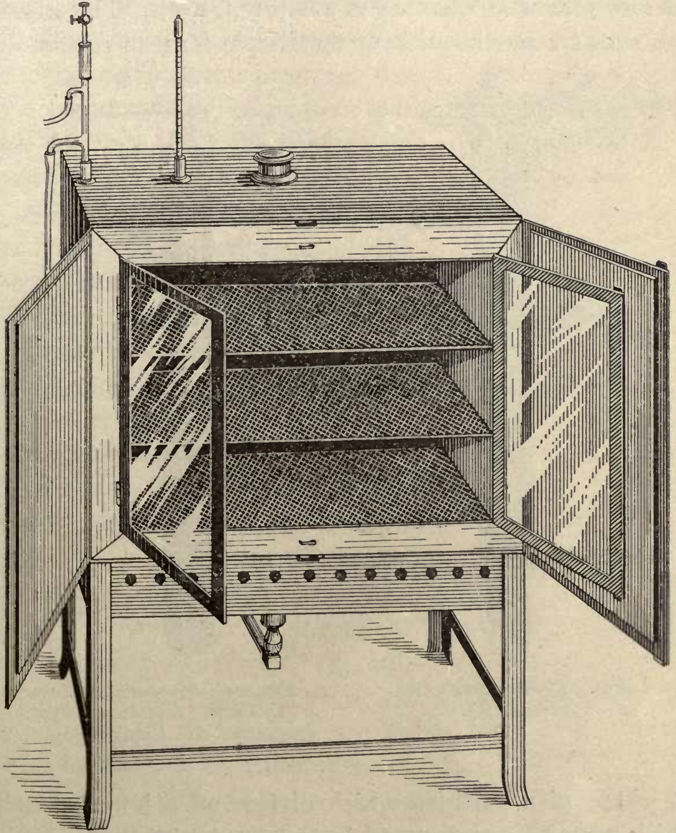

FIG. 22.—Incubator.

rises, and, little by little, cuts off the inflow of gas through this tube. The flow is never completely cut off, as there is a capillary opening in the tube considerably above any point to which the mercury could possibly rise, which will always allow the flow of a small quantity of gas (Fig. 25, *b*). This diagram also shows a modification of the simple form of regulator, in the shape of a partition which divides off a lower chamber, which contains mercury and is connected with the upper part by a glass tube. The purpose is to make use of the elastic properties of some volatile fluid, like ether, which floats on the surface of the mercury at *a*.

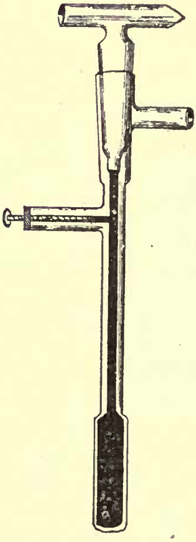

FIG. 23.—Reichert's Gas-regulator.

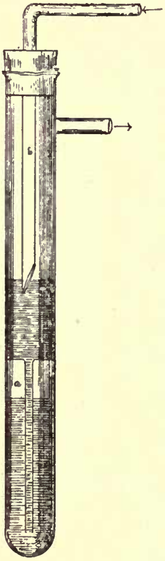

FIG. 24.—Mercurial Gas-regulator. *a*. Chamber containing volatile hydrocarbon. *b*. Capillary opening.

A most satisfactory gas regulator is that of Roux. It is constructed entirely of metal, and its operation is due to the unequal expansion and contraction of two metals which are riveted together. Fig. 25 shows this regulator. The gas passes in at *e* and passes out at *d*. The amount of gas passing through is regulated by a piston on the end of the set screw inside the tube from which the outlet tube branches off. This piston moves in or out according to the changes of temperature of the water jacket of the incubator into which the stem (*f*) of the regulator is inserted. This stem is finestrated and has the riveted metallic strips running down in it. These strips are pivoted at the collar, *g*.

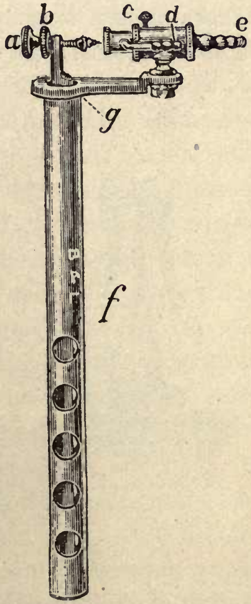

FIG. 25.—Roux Bimetallic Gas Regulator. *a*, Set screw; *b*, Screw collar; *c*, Clamp; *d*, Outlet for gas; *e*, Inlet for gas.

The gas coming from the gas-regulator passes to a Bunsen burner, which stands underneath the incubator. This burner should have some kind of automatic device for cutting off the flow of gas in case it becomes accidentally extinguished by a sudden draught of air or from any other cause. The automatic burner invented by Koch is an ingenious, simple and effective device (Fig. 26). The coils of metal seen on each side at the top of the burner are so arranged that when they expand they turn the disk below so as to support the arm coming from the stop-cock; when they cool they turn the disk in the opposite direction, and allow the arm to fall and cut off the gas. Some inconvenience will arise from irregularities in the flow of gas from the main supply-pipe. Any incubator will vary a little from such causes. In the experience of the writer, natural gas is of such variable pressure as to be entirely useless. Fluctuations of the temperature within the incubator depend very largely upon the external temperature. Therefore, the incubator should, as far as is practicable, be protected from sudden draughts of cold air and should be kept in a room having as equable a tempera ture as possible. In large laboratories it is often found convenient to use the whole of a small room as an incubator, heating it by a gas stove, to which a gas regulator may be applied.

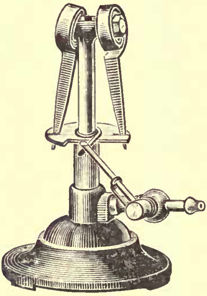
FIG. 26. Koch Automatic Gas-burner.

Culture-tubes which are being kept in the incubator are likely to become dry if their stay is prolonged. In such cases they should be covered with rubber caps, tin-foil, sealing-wax, paraffin or some other device to prevent evaporation. If rubber caps are used, they should be left in 1–1000 bichloride of mercury solution for an hour, and the cotton plugs should be singed in the flame before putting them on. (Fig. 21.) The writer prefers rubber stoppers, which may be boiled and stored in bichloride of mercury solution. In using stoppers in this way the cotton plug is cut even with the mouth of the tube, the top singed in the flame, and the plug shoved down into the tube for about 1 cm. The rubber stopper is then inserted (Fig. 20).

Low-temperature Incubator.—An incubator regulated for so-called "room temperature" is very desirable for the cultivation of bacteria upon gelatin and for the bacteriological analysis of water. In our climate the temperature of the rooms of the laboratory often reaches a point at which gelatin melts, and for this reason in a low-temperature incubator provision has to be made for cooling when the room temperature is too high as well as for heating when it is too low.

A form of incubator devised by Rogers* for this purpose consists of a refrigerator or of a specially constructed chamber heated by electricity and controlled by an electric thermoregulator. Below is given a description of an incubator constructed according to Rogers' plans. This incubator has been in use for some time and has given entire satisfaction since the precautions noted below were followed. There would appear no reason why this incubator should not be employed for high temperatures as well for low, but so far it has been run at 22° C. The temperature has kept very constant. The incubator consists of a refrigerator, 30 inches high, 24 inches wide, 18 inches from front to back, all outside measurements. Instead of the ordinary drip pipe, there is a coil of 1-inch galvanized iron pipe run down the back of the cooling chamber attached water-tight to the ice tank. From the bottom of the cooling chamber the coil runs up perpendicularly nearly to the bottom of the ice compartment, and then runs horizontally through the wall of the refrigerator. A bracket on the outside supports a drip-pan. A thermometer encased in a finestrated metal jacket is inserted about half way up on one side. A lump of ice, about 50 pounds, placed in the ice compartment serves to keep the temperature sufficiently cool. In summer doubtless more ice will be required.

For heating, an ordinary, 16-candle-power electric bulb is used, and the electricity is obtained from the public supply. The wire is run through one of the walls, and a part of the current is made to operate a horse-shoe magnet, and another part is conducted through the lamp used for heating.

The accompanying diagram, (Fig. 27), will serve to show the arrangement.

A telegraph key is used to supply the horse-shoe magnet

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*L. A. Rogers. On electrically controlled low temperature incubators. *Centralblatt für Bakteriologie*, etc. Bd. XV. Abt. II. Double No. 7–8. pp. 236–239. Sept. 23, 1905.

which is inserted in the heating circuit in such a way that when the armature is attracted toward the magnet the circuit is completed and the lamp is consequently lighted. The part of the current, *a*, supplying the magnet first passes through a small lamp and through two resistance coils so as to reduce the pressure. It then passes through the magnet, and is continued on to the set-screw, *b*, which is so placed that when the thermoregulator comes in contact with it the current is complete. The regulator consists of a strip of hard rubber and a strip of brass riveted together. One end is fixed, while the other is free, and when it is heated it tends to bend toward the metal side, when it cools it bends toward the rubber. The brass strip is 15 inches long, $\frac{1}{8}$ inch thick, and $\frac{1}{2}$ inch wide; the rubber strip is $\frac{1}{4}$ inch thick, $\frac{1}{2}$ inch wide, and a little less than 15 inches long. In the diagram the end is fixed at *d* and is free at *b*. When it is heated, the free end travels away from the set-screw at *b*; when it cools, it moves toward the set-screw. Rogers also recommends a regulator made of invar and

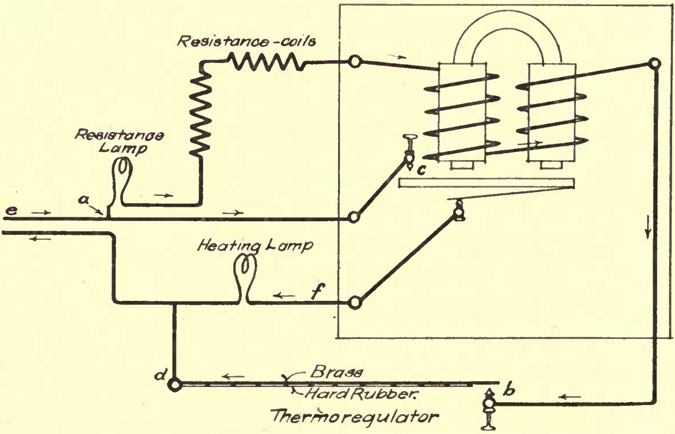

FIG. 27.

brass instead of hard rubber and brass. Where invar is used instead of the hard rubber the dimensions for the two metals are the same as those given for the brass strip in the hard rubber-brass regulator just described. As is evident from the description, the circuit controlling the magnet is closed whenever the free end of the regulator comes in contact with the set screw at *b*. When this circuit is closed the magnet attracts the armature, and the heating circuit is closed by the contact formed at *c* between the armature and the set-screw. In the diagram this point of contact is put to one side for the sake of clearness, but as a matter of fact in the instrument in use the set-screw is above and between the ends of the horseshoe magnet, and comes in contact with the armature which is extended upward in the shape of a tongue. From the description just given it will be noted that the thermoregulator does not regulate the heating directly, but indirectly through the electro-magnet.*

Certain precautions have been found necessary in practice in order to obtain satisfactory results with this incubator. The set-screw against which the armature strikes at *c* should be so set that the armature does not come in contact with the magnet. In the apparatus described above there is a space of about ⅛ inch between the armature and the magnet when contact takes place between the set-screw and the armature. If the set-screw does not project far enough to prevent the armature from coming in contact with the magnet the armature is apt to stick to the magnet even after the current is broken at *b*, and when this is the case of course the lamp remains lighted, and the temperature may run up too high. This sticking of the armature to the magnet is said to be due to the residual magnetism left in the core of the magnet. When the current passing through the magnet is broken by the excursion of the end of the thermoregulator away from the set-screw at *b*, the armature is pulled away from the magnet by a coiled spring. Another important precaution is that the points at which contact is made and broken, *b* and *c*, should be tipped with platinum. A small piece of platinum wire inserted into the ends of the set-screws and a few square centimeters of platinum foil riveted to the opposite point of contact meets the requirements. If platinum is not used at these points oxydation takes place and prevents contact. The set-screw at *b* is set by experiment for the temperature desired. The further the point of the set-screw projects toward the free arm of the regulator, the higher the temperature maintained.

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* Recently the regulator has been connected directly with the current passing through the heating lamp without the intervention of the horse shoe magnet, connecting the wires *e* and *f* directly with *d* and *b*.

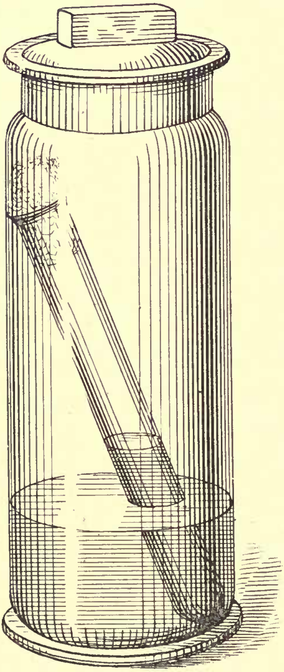

FIG. 28.—Arrangement of tubes for cultivation of anaërobes by Buchner's method.

CULTIVATION OF ANAEROBIC BACTERIA.

The cultivation of anaërobic bacteria is done best in a medium containing 1 to 2 per cent. of dextrose. The tube should contain a large quantity of the culture-medium. Just before using, the medium should be boiled for a few minutes. Inoculate the tube after cooling, but while the medium is fluid. Anaërobes may be cultivated in the closed arm of the fermentation-tube (see Fig. ~~46~~ 48), but the opening between the two arms of the tube must be small.

Buchner's Method for the Cultivation of Anaërobes.—Into a bottle or tube which can be tightly stoppered, pour 10 c.c. of a 6 per cent. solution of sodium or potassium hydroxide, for each 100 c.c. of air contained in the jar. Add one gram of pyrogallic acid for each 10 c.c. of solution. The culture-tube is placed inside of the larger bottle or tube, supported above the bottom, and the stopper, smeared with paraffin, is inserted. The mixture of pyrogallic acid and potassium hydroxide possesses the property of absorbing oxygen.

Wright's Modification of Buchner's Method.—The tube of culture-medium is to be plugged with *absorbent cotton*, using a plug of large size. The culture-medium is inoculated in the usual way. The plug is cut off close to the neck of the tube, and is then pushed into the tube about 1 centimeter. Now allow a watery solution of pyrogallic acid to run into the plug, and then a watery solution of sodium or potassium hydroxide. Close quickly and tightly with a rubber stopper. Wright recommends that the first solution be freshly made and consist of about equal volumes of pyrogallic acid and water, and that the second solution contain 1 part of sodium hydroxide and 2 parts of water. With 6-inch test-tubes, ¾-inch diameter, the amounts advised are—½ c.c. solution of pyrogallic acid, 1 c.c. solution of sodium hydroxide.

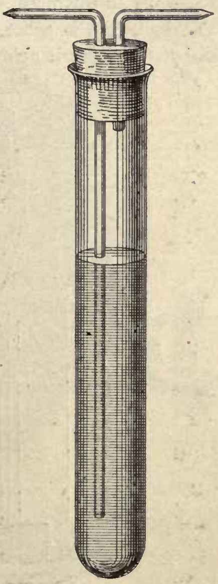
FIG. 29.—Cultivation of anaërobes by Fränkel's method.

Cultivation of Anaërobic Bacteria under Hydrogen: Method of Liborius Modified by Fränkel.—A test-tube containing a large amount of the liquefied culture-medium is closed with a sterilized rubber stopper, through which pass two sterilized glass tubes, bent above the stopper at a right angle. One of these tubes is cut off just underneath the stopper, and the other is long enough to project nearly to the bottom of the culture-tube. The horizontal projecting parts are drawn to a small caliber at some point, although not quite closed, to facilitate sealing later on. Through the longer of these tubes hydrogen gas is passed until the atmosphere inside of the culture-tube is pure hydrogen, entirely free from mixture with air. The horizontal parts of the small glass tubes projecting from the stopper are then sealed in the flame at the places where they were previously drawn out to a small caliber, and the tubes are thus closed. (Fig. 27.)

The stopper should be surrounded with melted paraffin. A tube prepared according to this plan may, if desired, be converted into an Esmarch roll-tube. The hydrogen is generated according to the common method with *pure* zinc and *pure* sulphuric acid, 25 to 30 per cent. The precautions advised by chemists for the generation of hydrogen must be carefully followed because when hydrogen mixed with oxygen or air is ignited a violent and disastrous explosion may occur. Those unfamiliar with the generation of hydrogen should seek the aid of someone who is informed so as to avoid accidents arising from explosion. The hydrogen must not be allowed to escape in the neighborhood of a flame for fear of explosion.

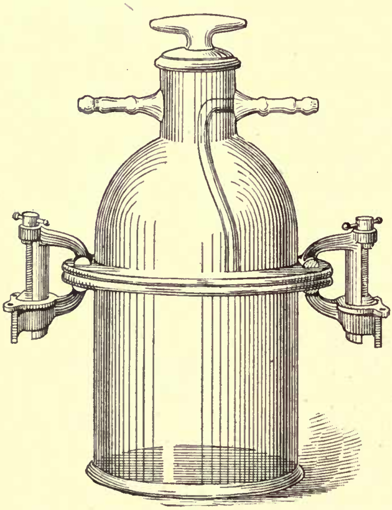

FIG. 30.—Novy's jar for the cultivation of anaërobes.

The well-known Kipp's generator may be used. First let the reservoir fill with hydrogen; then allow its contents to escape. This should be repeated, after which some of the hydrogen may be collected in an inverted test-tube under water. When this sample is ignited, it should burn without any explosion; for if it explodes this shows that oxygen is still mixed with it. The hydrogen should bubble through the medium five minutes or more.

The inconvenience and danger of sealing the tubes in the flame, as has to be done in Liborius-Fränkel's and other methods for cultivation under hydrogen, are obviated in *Novy's apparatus.* The tubes or plates are placed in jars through which hydrogen may be conducted. The stopper, having been smeared previously with a soft wax, is sealed by giving it one-fourth of a turn.

There have been various other kinds of apparatus, usually complicated and expensive, devised for the growth of plate-cultures under hydrogen, but Novy's jars are the best, both for tubes and for plates.

Other expedients for the cultivation of anaerobic bacteria are less effective.

In cases where a very deep stab-culture is made in gelatin or agar, where the growth appears in the lower part of the tube by preference, it is supposed to be anaerobic. Koch covered part of the surface of a gelatin plate with a bit of sterilized mica or a cover-glass; bacteria which grew beneath this place were considered to be anaerobic. Another method was to cover the surface of the gelatin in the culture-tube with sterilized oil. W. H. Park has recommended a mixture of solid paraffin with 25 to 50 per cent. of fluid paraffin or albolene as a covering for the surface of anaerobic cultures. This mixture has a semi-solid consistency, and does nor retract at the edges on cooling. The paraffin prevents the absorption of oxygen except to a small extent at the edges. The method is useful for large quantities of culture material, as in flasks. Esmarch advised making roll-tubes, and after cooling them to fill them with a melted gelatin cooled down to near the point of solidification. Hueppe made use of eggs in their shells. The egg-shell was carefully cleaned, sterilized with a solution of bichloride of mercury, washed with sterilized water and wiped dry with sterilized cotton. The end of the egg-shell was punctured with a hot needle. Through the opening thus made the inoculation was accomplished. The opening was closed with collodion.

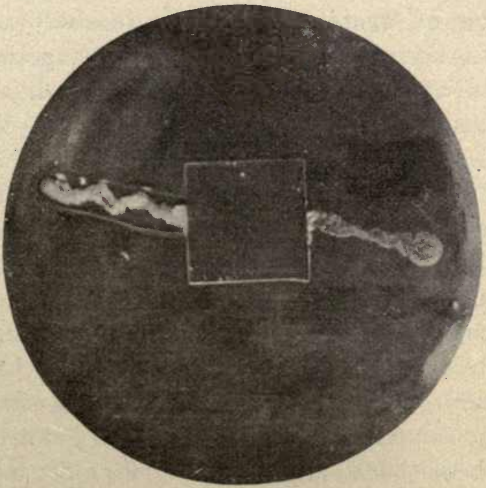

FIG. 31.—Streak culture of the potato bacillus (natural size), showing an aërobic organism which will not grow under a cover-glass.

CHAPTER V.

CULTIVATION OF BACTERIA, CONTINUED.

Isolation of Bacteria.—In order to study any kind of bacteria it is necessary to have the particular species separated from other sorts with which it may be mixed. The earlier bacteriologists endeavored to separate bacteria of different sorts of successive transplantations through a series of tubes. The procedure now generally used for this purpose is the so-called plate-method of Koch. The great progress which bacteriology has made during the last twenty years is largely owing to the use of this method.

Pathogenic bacteria may sometimes be isolated through inoculations into animals. Thus an animal may be inoculated with sputum containing tubercle bacilli mixed with other bacteria. The animal may die of tuberculosis, and its tissues may contain tubercle bacilli in pure culture, the other bacteria having produced no important effect.

Still another method which is occasionally useful is to subject the mixture of bacteria to a heat of 80° or 90° C. for a few minutes. If it contains resistant spores, like those of the tetanus bacillus or hay bacillus, they may be expected to survive, and may be propagated in pure culture, everything else having been killed by the heat.

Plate-cultures.—It is impossible in most cases to distinguish between bacteria of different varieties by microscopic examination alone. Bacteria of widely different species and quite unlike one another in their properties may present similar appearances under the microscope. The differences which they exhibit are usually apparent when they are grown in culture-media. The growth, called a *colony*, which results from the multiplication of a single bacterium, is in many cases quite characteristic for the species. By the plate-method the individual bacteria in a mixture are separated from one another by distributing them through melted gelatin or agar in tubes. They are fixed in the place where they chance to be when the medium solidifies. They are allowed to grow, and from each individual there forms a colony. It is usually possible to distinguish between *colonies* arising from different species when it was not possible to distinguish between *the individual bacteria* of these species. A convenient illustration has been suggested by Abbott. A number of seeds of different sorts may appear very much alike, and considerable difficulty may be found in distinguishing one from another with the eye. Let them be sown, however, and let plants develop from them, and these plants will easily be distinguished from one another.*

Method of Making Plate-cultures.—Melt *three* tubes of gelatin or agar. (There is some difficulty in keeping agar in a fluid state while dilutions are being made. It is best to have some form of water-bath with a thermometer for the purpose.) Let the melted tubes cool to a few degrees above 40° C. Take a small portion of the material to be examined—pus, for example—and introduce it with a sterilized platinum wire or loop into one of the tubes. Stir it in carefully. Remove the needle, sterilize it and replace the plug. Mix the material introduced thoroughly with the melted culture-medium, taking care not to wet the plug. Now remove the plug again, and, having sterilized the platinum wire, insert it into the liquefied medium. Carry three loopfuls in succession from this tube, which is No. 1, into tube No. 2; sterilize the needle; replace the plugs; mix thoroughly by tilting the tube up and down, but avoid shaking

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*It must be understood that *no close comparison can be drawn between higher plants*, which simply complete the development of parts potentially present in the seed, *and colonies of bacteria*, which are aggregates of individuals the progeny of one individual of the same kind.

the medium up into foam, and also avoid wetting the plug. Carry three loopfuls from tube No. 2 into tube No. 3 in the same manner. The original material will obviously be diluted in tube No. 1, more in tube No. 2 and still more in tube No. 3. The most convenient form of plate is that known as a Petri dish, a small glass dish about 8 cm. in diameter and 1.5 cm. in height, provided with a cover which is a little larger, but of the same form. This dish should be cleaned and sterilized in a hot-air sterilizer at 150° C. or higher for an hour. When it is cool it may be used.

Such dishes having previously been prepared, the contents of tube No. 1 are poured into one dish, and those of tube No. 2 into another and those of tube No. 3 into a third. They are to be labeled Nos. 1, 2 and 3.* In pouring proceed as follows: Remove the plug of tube No. 1; heat the neck of the tube in the flame; allow it to cool, holding it in a nearly horizontal position. When the tube has cooled, lift the cover of the Petri dish a little, holding it over the dish; pour the contents of tube No. 1 into the dish and replace the cover of the dish. The interior of the dish should be exposed as little and as short a time as possible. Tubes Nos. 2 and 3 are to be treated in the same manner. Burn the plugs, and fill the empty tubes with 5 per cent. solution of carbolic acid. They should be sterilized for an hour in the steam sterilizer on each of three days.

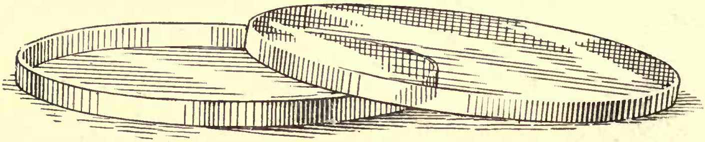

FIG. 32.—Petri dish.

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*The labels should be moistened with the finger, which has been dipped in water. They should not be licked with the tongue. While working in the bacteriological laboratory it is best to make it a rule that no object is to be put in the mouth.

The culture-medium in the Petri dish will soon solidify. Petri dishes of agar should be inverted as soon as the medium is thoroughly solidified, otherwise the water which evaporates

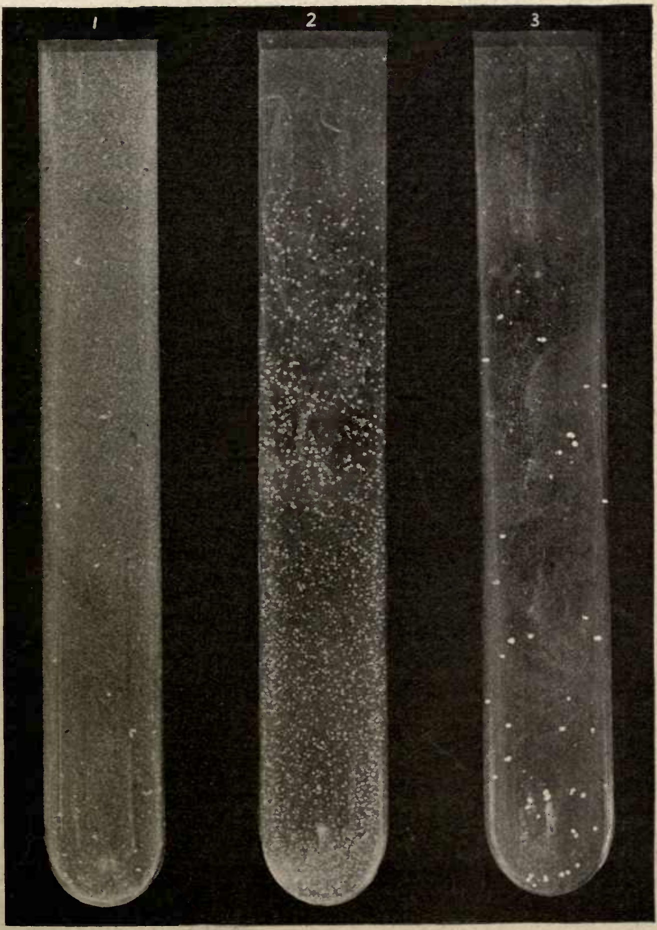

FIG. 33.—Dilution-cultures in Esmarch roll-tubes.

In tube 1 the colonies are very close together; in tube 2 they are somewhat separate; in tube 3 they are well isolated.

from the surface condenses on the inside of the lid, and runs down over the surface of the agar. A round piece of filter paper placed over the dish before putting on the lid may also be employed, or the cover may be made of porous earthenware, as recently recommended by Hill. Colonies develop usually in from one to two days, more quickly, of course, in the incubator. In plate No. 1 they will be very numerous, in plate No. 2 less numerous and in plate No. 3 still less numerous. Where the number is small the colonies will be widely separated and can readily be studied. They may be examined with a hand-lens, or the entire dish may be placed on the stage of the microscope and the colonies be inspected with the low power. The iris diaphragm should be partly closed and the concave mirror should be used. Dilution-cultures prepared as described in the next paragraph, where the principle is the same, are shown in Fig. 31. In tube No. 1 the colonies are so numerous as to look like fine, white dust. In tubes 2 and 3 they become less numerous and larger.

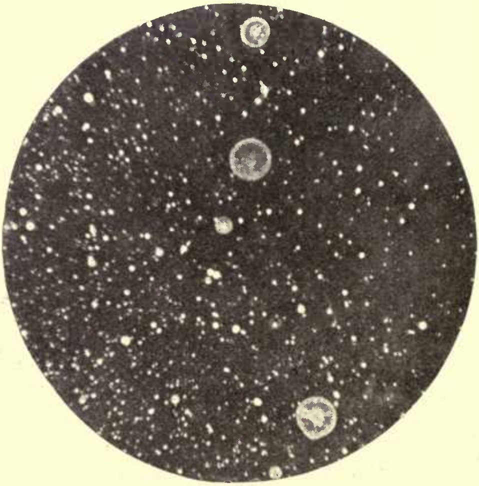

FIG. 34.—Appearance of colonies on gelatin in Petri dish.

Esmarch's Roll-tubes.—Either gelatin or agar may be used for roll-tubes, but if the agar is freshly made, it does not adhere well to the walls of the tuber. The dilutions in tubes 1, 2, and 3 are made as above. Tubes containing a rather small amount of the culture-medium are more convenient. The tubes may be capped with a rubber cap and revolved in a dish of ice water, or under the water tap or, a still better method employed first by W. D. Booker, a block of ice should be at hand, and, with a tube filled with hot water and lying horizontally, a hollow of the size of the test-tube should be melted on the upper surface of the ice. In this hollow place the tube of liquified gelatin or agar; roll it rapidly with the hand, taking care that the culture-medium does not run toward the neck as far as the cotton plug. The medium is spread in a uniform manner around the inside of the tube, where it becomes solidified. Gelatin roll-tubes must be kept in a place so cool that there is no danger of their melting; in handling them they are to be held by the portion of the tube into which the cotton plug projects, so that the warmth of the hand may not

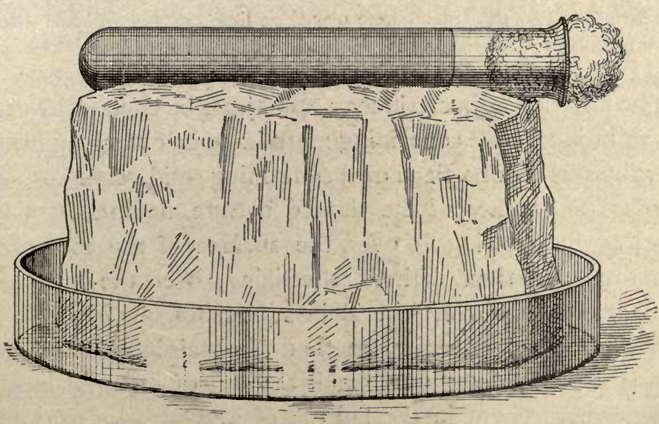

FIG. 35.—Manner of making Esmarch roll-tube.

melt the gelatin. Agar roll-tubes should be kept in a position a little inclined from the horizontal, with the mouth up, for twenty-four hours, so that the agar may stick to the wall of the tube. For reasons stated above, it is best to employ agar which has partially dried out.

By the plate-method as originally devised by Koch, instead of using Petr dishes, the gelatin was poured upon a sterile plate of glass. This plate of glass was laid on another larger plate of glass, which formed a cover for a dish of ice-water, the whole being provided with a leveling apparatus. The plate was kept perfectly level until it had solidified, which took place rapidly on the cold surface. The glass plates were placed on little benches enclosed within a sterile chamber. This method is now seldom or never used.

The isolation of bacteria may sometimes be effected by drawing a platinum wire containing material to be examined rapidly over the surface of a Petri dish containing solid gelatin or agar; or over the surface of the slanted culture-medium in a test-tube; or by drawing it over the surface of the medium in one test-tube, then without sterilizing, over the surface of another, perhaps over several in succession.

Another very convenient method of obtaining isolated colonies is to introduce a very small amount of material into the water squeezed out in the bottom of a slant agar tube, then flood this over the surface of the agar.

Appearance of the Colonies.—The colonies obtained in the Petri dishes or roll-tubes (Fig. 32) may be studied with a hand-lens or with a low-power microscope. In the latter case, use the concave mirror with the iris diaphragm partly closed. The colonies present various appearances. Some of them are white, some colored; some are quite transparent and others are opaque; some are round, some are irregular in outline; some have a smooth surface, others appear granular, and others present a radial striation. Surface colonies often present different appearances from those occurring more deeply. Surface colonies are likely to be broad, flat and spreading. If the colony consists of bacteria which have the property of liquefying gelatin, a little funnel-shaped pit or depression forms at the site of the colony. The appearance of colonies may be of great assistance in determining the character of doubtful species. The appearance in gelatin plates of the colonies of the spirillum of Asiatic cholera, for instance, is one of the most characteristic manifestations of this organism.

Pure Culture.—From these colonies pure cultures may be obtained by what is called "fishing." Select a colony from which cultures are to be made; touch it lightly with the tip of a sterilized platinum wire, taking great care not to touch the medium at any other point. Introduce the wire into a tube of gelatin. Sterilize the wire and plug the tube. In a similar manner, and from the same colony, inoculate tubes of agar, bouillon, milk, potato and blood-serum. At the same time it is well to make a smear preparation from the colony and to stain with one of the aniline dyes so as to determine the morphology of the bacteria. The growths which take place in the tubes should contain one and the same kind of bacteria. As seen under the microscope the bacteria should have the same general form and appearance as those seen in the colony from which they were derived. This will be the case, provided the colony has resulted from the development of a single bacterium or from several bacteria of the same kind. Occasionally, however, a colony will develop from several bacteria which may not all be alike. In that case a pure culture will not be obtained, and the process of plating may have to be repeated.

CHAPTER VI.

INOCULATION OF ANIMALS.

In the study of pathogenic bacteria, the inoculation of animals is frequently indispensable. It is inexpedient where classes are large for students to make such inoculations; but, nevertheless, every student should be familiar with the subject. The animals most often used are white mice, guinea-pigs, rabbits and pigeons. Larger animals are also employed for special purposes. The hair in all cases should be removed from the point selected for inoculation, and if the material to be introduced is solid or semisolid, as with tissue pulp, a small **V**-shaped opening in the skin is made with scissors, and a stiff, sterilized, platinum wire or with the point of the scissors or of a pair of forceps, is passed into this opening, separating the skin from the muscles for some distance so as to make a pocket. Into this pocket the material is introduced by means of the platinum wire. The wound may be covered with collodion. Such solid or semi-solid material may also be conveniently introduced by placing them in a sterile glass cannula, which is pushed to the proper situation through a small incis-

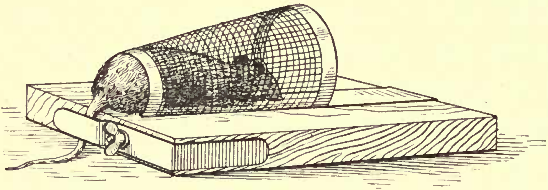

FIG. 36.—Mouse-holder.

ion. The substance in the cannula is forced out of it with a stiff, sterile, platinum wire. (Fig. 37.)

The peritoneal cavity may be inoculated through an incision in the abdominal wall, into which the desired substance may be introduced with a sterile platinum wire, the incision being closed with sutures.

But a more convenient method in many cases, both for subcutaneous as well as intraperitoneal inoculations, is the use of a hypodermatic syringe. Material from the surface of solid media can be suspended in sterile beef-broth or physiological salt solution, or tissue pulp may be mascerated in the same liquids, or cultures in fluid media used directly for these injections.

Intravenous inoculation is most commonly practiced upon rabbits. A small vein which is near the posterior margin of the ear of the rabbit is easily reached from the dorsal surface; the ear having been shaved and washed with alcohol, the hypodermatic needle is introduced directly into this vein. In making a hypodermatic injection, the needle and syringe should of course be sterilized before and after each operation.

For the inoculation of mice resort may be had to some sort of mouse-holder (Fig. 34), or the animal may be held by an assistant, who takes the skin at the back of the neck between his fingers and at the same time holds the tail. Resort may be had to etherization by using a few drops of the anesthetic on a bit of cotton wool placed in the jar with the mouse, but there is danger of killing the animal in this way. After inoculation the mice may be kept in a glass jar covered with

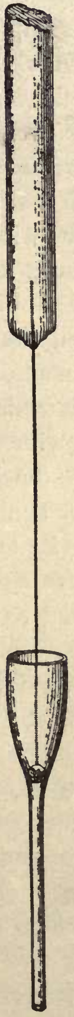

FIG. 37.

wire netting or in a Mason preserve jar with holes punched in the top. They may be fed with moistened bread or oats. It is important to see that they receive drinking-water.

Guinea-pigs and rabbits may be held by an assistant or tied by the legs upon a board for purposes of inoculation. The hair over a small portion of the abdomen is cut away and a short incision is made through the skin; a pocket is produced with a stiff wire, and the material inserted with a sterile platinum wire. The wound may be covered with collodion. Sutures may be used if the wound is large. Guinea-pigs and rabbits, after inoculation, are to be kept in cages of galvanized iron and wire-netting. The bottom may conveniently be made in the form of a movable pan which permits of the disinfection of the excreta. Rabbits and guinea-pigs may be fed with oats, carrots, cabbage, grass and the like.

Autopsies upon animals should be held as soon as possible after death. During the interval the body should be kept in the ice-box. The autopsy-room should be furnished with screens to keep out flies, so that they may not light on the infected animal. The animal should be extended on its back upon a board. The legs may be fastened with pins or tacks. The animal should be handled with forceps as far as possible, and after beginning the autopsy the fingers should not touch it. If the fingers come in contact with infectious matter, disinfect them at once. Have a basin of bichloride of mercury solution 1–1000 ready for this purpose. Knives, scissors, platinum wires and forceps should be sterilized in the flame before and after each manipulation. Be prepared to make smear preparations on cover-glasses, and to inoculate tubes of gelatin, agar and other media as desired. Moisten the hairs over the thorax and abdomen with bichloride of mercury solution—1000, to prevent them from being carried into the air. Make an incision, passing through the skin from the sternum to the pubis along the thorax and abdomen, and diagonal incisions extending down the fore and hind legs. Dissect away the skin from the thorax, abdomen and upper parts of the legs. With a knife heated in the flame, sear a broad line extending down the middle of the abdomen. Through this burned surface make an incision through the muscles of the abdomen. In a similar manner make a transverse incision across the middle of the abdomen through a burned surface. Inoculations on culture-media should be made from the peritoneal cavity, cover-glass preparations which are afterwards to be stained should be made by smears from various tissue juices. With a hot knife, scorch a small area on the surface of the liver; through this surface enter the liver with a sterilized platinum wire, and with the material thus obtained inoculate the tubes; also make cover-glass preparations. In the same manner inoculate tubes and make cover-glass preparations from the spleen, the kidneys, the pleural cavity, the pericardial cavity, the lungs, and the blood inside the heart and other organs as indicated. If there is a question of the tissues from which the cultures are to be made having become contaminated, as might be the case where the autopsy is delayed for any reason, it is better to make plate cultures in Petri dishes as described on page 96. All incisions are to be made through the burned surfaces, and all material used for inoculation is to be obtained through burned surfaces. In sterilizing the instruments in the flame avoid sputtering, especially when they become covered with oil from adipose tissue. Pieces of lung, liver, spleen, kidney and other organs, as may be indicated, should be placed in 95 per cent. alcohol or 10 per cent. formalin for fixation and hardening. The animal and the board on which it was extended should be covered with bichloride of mercury solution 1–1000, and afterward burned. The cage or jar and the instruments, dishes and towels used should be sterilized by steam. The hands of the operator should be washed thoroughly with soap and water and with a 1–1000 solution of bichloride of mercury, if there is any possibility of these having accidentally come in contact with any of the diseased tissues.

Collodion Capsules.—Bacteria may be cultivated in the living body of an animal, without infecting the animal, when they are enclosed in collodion capsules. Their soluble products are able to diffuse through the collodion, while the animal's fluid may pass into the sac to nourish them. These capsules were originally made by dipping the round end of a glass rod into collodion repeatedly. McCræ's method* is easier and more satisfactory. (Fig. 36.)

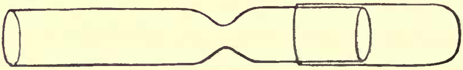

FIG. 38.—Method of making collodion capsules.—(*After McCrae.*)

A piece of glass tubing is taken, and a narrow neck drawn on it near one end. This end of the tube is rounded in the flame and while still warm, the body of a gelatin capsule is fitted over it, so that the gelatin may adhere to the glass. The capsule is now dipped into 3 per cent. collodion, covering the gelatin and part of the glass. It is allowed to dry a few minutes, and is dipped again. In all, two or three coatings may be given. The capsule is filled with water and boiled in a test-tube with water. The melted gelatin is removed from the inside of the capsule by means of a fine pipette. The causule is partly filled with water or broth and sterilized. The capsule may now be inoculated. The narrow part of the glass tube which constitutes the neck must then be sealed in the flame, taking care that the neck be dry. The sealed capsule should be placed in bouillon for twenty-four hours. No growth should occur outside the capsule if it is tight. It may now be placed in the peritoneum of an animal.

A method for making collodion sacs recommended by Gorsline† consists in the use of a glass tube, the lower end of which is rounded and closed, except a small hole, which is temporarily filled with collodion. This tube is dipped in collodion and dried, as above. It may now be filled with water. By blowing at the opposite end, the pressure through the hole in the bottom of the glass tube will cause the capsule to loosen so that it comes away easily.

There are also various other methods recommended for making collodion sacs.

***Journal of Experimental Medicine.* Vol. VI., p. 635.

†See Contributions to Medical Research. Dedicated to Victor C. Vaughan, 1903.

CHAPTER VII.

COLLECTION OF MATERIAL.

All material used for bacteriological examination should be fresh, for the reason that changes in the number as well as the kinds of bacteria take place quickly in such material as furnish a suitable soil for the development of bacteria under ordinary circumstances. Samples of water or milk should be examined as soon after drawing as possible; but when this is impossible, as in the case where they are transmitted from a distance, they should be collected in sterilized tubes or bottles, which should be kept on ice but not frozen. Specimens of sputum should be collected in clean bottles tightly corked. The early morning sputum is to be preferred for examination. The patient should be directed to rinse out the mouth carefully, and cough up material from the lungs, not merely to clear out the throat as is sometimes done. It should be examined as soon as possible. Although decomposition appears not to interfere with the staining properties of the tubercle bacilli, the sputum should be fresh in order that the other bacteria contained in it may be studied. Therefore, it should be free from contamination with putrefactive germs. Valuable information can also be obtained by examination of sputum in a fresh condition before staining (see also page 34).

Samples of urine keep better after the addition of a few crystals of thymol, which retards the fermentative process, so that the sedimentation of the bacteria and of other solid matter in conical vessels is facilitated, although that purpose can be accomplished at once by the centrifuge. Thymol will also be a useful addition, as far as a bacteriological examination is concerned, in case samples of urine are to be sent by mail; thymol should not be added if cultures are to be made.

Specimens of sputum, pus or blood may be collected conveniently in the form of thin smears upon cover-glasses. The smears are fixed by passing through the flame three times. Smears of blood are prepared as follows: Have two perfectly clean, square cover-glasses. The finger, or the lobe of the ear, having been carefully washed with water, alcohol and ether, is punctured with a sterilized needle, and a small drop of blood issues which is wiped away with a clean cloth. The second drop of blood should be taken; it should be about the size of a pin's head. No pressure should be exerted upon the skin. This drop of blood is placed on one of the cover-glasses. The other cover-glass is laid upon the first, both being handled with forceps. The drop of blood becomes flattened out into a thin film. Immediately and before the blood has had time to coagulate the two are slipped or slid away from each other in a horizontal plane, not forcibly pulled apart. In this way the blood will be spread in thin films on the cover-glasses. It is best to place the cover-glasses so that one does not cover the other exactly, but so that the sides of the one lie diagonally to the sides of the other, although their centers coincide (Fig. 37). Films of blood which are to be examined for the parasite of malaria may be prepared in this manner. Drops of blood to be used for the serum reaction for typhoid fever must be large-sized. They may be collected on cover-glasses or pieces of unsized paper and allowed to dry. In place of drops of blood caught in this manner, the use of capillary tubes for collecting the blood are to be preferred, since by this method it is possible to obtain the serum free from corpuscles after the clot forms in the tube. To test blood by culture methods

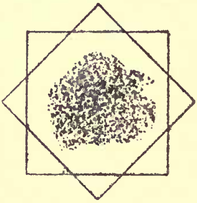

FIG. 39.—Manner of placing cover-glasses in making films of blood.—(*After Cabot.*)

not less than 10 c.c. of blood should be taken.* This is most conveniently accomplished by using a large hypodermatic needle, and aspirating the blood from a vein at the bend of the elbow—under strict antiseptic precautions. A bandage tied tightly around the arm above the elbow facilitates the operation. The blood thus taken may then be used for cultures in various ways. A good method for general purposes is to empty the syringe quickly into a flask holding 100 c.c. or more of bouillon or dextrose-bouillon. The mixture of blood and bouillon should be placed in the incubator for one to two days. If the the bacteria develop, they may be secured in pure cultures by plating, and may be studied further, as the occasion requires.

At autopsies on human subjects the same principles apply as in the case of autopsies upon animals (see pages 104 and 109). Plate-cultures should be made, if possible, directly from the organs. In all cases organs should be seared with a hot spatula over the point where the platinum wire is inserted. The method of isolation by streaking the platinum wire containing the material under examination lightly, several times, over the surface of an agar plate, will be found convenient. A still more convenient method is to inoculate the water which is collected at the bottom of a stout agar tube and flood it over the surface of the agar. In the tubes inoculated in this isolated colonies grow out on the surface of the slanted agar. At the same time smears should be made from the organs upon cover-glasses for microscopic study, and portions of the organs should be saved and hardened in alcohol or formalin.

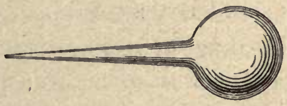

FIG. 40.—Sternberg bulb.

A convenient device for the collection of infected material is a stiff wire wound with a pledget of absorbent cotton at one end,

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*For examination of blood for typhoid bacilli see Libman. Bul. *Johns Hopkins Hosp.* Vol. XVII., July, 1906.

the whole sterilized in a tube, as recommended by Warren for collecting pus and other fluids for examination, and as introduced by W. H. Park for the collection of material from the throat in cases of suspected diphtheria (Fig. 83).

The so-called Sternberg bulb* is valuable for the collection of fluid materials for examination. A piece of glass tubing is taken and drawn out to a long, fine tube, and a bulb blown at the other end. To introduce the substance into the bulb, the expanded end is heated in the flame; the point introduced below the surface of the fluid which is to be collected; as the bulb cools, the air in it contracts and draws the fluid into it. When it has taken up as much as it will, the point may be sealed off in the flame. If it is to sent to a distance and the same precautions should be used by those performing the autopsy to guard against becoming infected. The hands should be protected with rubber gloves. It should be so packed that breakage or leakage is impossible, particularly when infectious material is to be transported.

Concerning the transmission of materials containing bacteria in the mails, the ruling of the post-office department of the United States, March 2, 1900, is a follows:

"That the order of the Postmaster General of December 27, 1897 (Order No. 677), amendinng Order No. 88 of February 5, 1896, prescribing the conditions under which specimens of diseased tissues may be admitted to the mails is hereby further modified in the following manner:

"Specimens of diseased tissues may be admitted to the mail for transmission to United States, State, or municipal laboratories, only when enclosed in mailing packages constructed in accordance with the specifications hereinafter enumerated: Liquid cultures, or cultures of microörganisms in media that are fluid at the ordinary temperature (below 45° C. or 113° F.) are unmailable. Such specimens may be sent in media that remain solid at ordinary temperatures.

"Upon the outside of every package shall be written or printed the words 'Specimen for Bacteriological Examination. This package to be treated as letter mail.' No package containing diseased tissue shall be delivered to any representative of any of said laboratories until a permit shall have first been issued by the Postmaster General certifying that said institution has been found to be entitled, in accordance with the requirements of this regulation, to receive such specimens."

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*These bulbs were first recommended by Flügge. Die Mikroöganismen. 1 Auflage, p. 662. 1886.

The regulation includes not only cultures, but "specimens of diseased tissues." The specifications prescribing the manner of packing, which are minute and complicated, may be obtained from local postmasters.

CHAPTER VIII.

SYSTEMATIC STUDY OF SPECIES OF BACTERIA.*

In order to conduct the study of any species of bacteria it is necessary to have the organism isolated in a pure culture. This is best accomplished by the plate method already described. Having thus obtained the organism in pure culture, it is to be examined with reference to its behavior in certain particulars. It is well for the beginner to study a few known species of saprophytes obtained from some reliable laboratory in pure culture. The points which are to be considered can be illustrated best by presenting them in tabular form, filling out the items of the table for a given species of bacteria.

1. Name.
2. Habitat or source.
3. Morphology; grouping, as in chains or in zoöglœæ.
4. Size.
5. Staining properties. Behavior by Gram's method.
6. Capsule, present or otherwise.
7. Spore formation.
8. Motility, flagella.

Observations as to morphology, grouping, size, staining properties, and motility should all be made on fresh cultures. Agar cultures from 18 to 24 hours old are usually selected for the purpose. This rule cannot always be adhered to from the nature of the case as, for example, in the examination of cultures of the tubercle bacillus, of the gonococcus, of the diphteria bacillus. Examination for the presence of spores may also require older cultures.

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*See Appendix I., for recommendations of Society of American Bacteriologists.

Growth on culture-media.

9. Relation of growth to temperature.
10. Gelatin; observe whether the gelatin is liquefied or not. Colonies in gelatin plates, study under low power of microscope.
11. Agar. Colonies in agar plates, study under low power of microscope.
12. Bouillon; note cloudiness, pellicle or precipitate.
13. Milk; observe the reaction and whether or not the milk is coagulated and subsequently peptonized.
14. Production of gas in fermentation-tube with bouillon containing sugar, as dextrose, or in agar with sugars.
15. Potato.
16. Blood-serum; observe whether or not peptonization occurs.
17. Production of indol.
18. Pigment formation.
19. Production of acid or alkali.
20. Relation to oxygen; observe whether the superficial or the deep part of the growth is the more luxuriant in stab-cultures; use anaerobic methods if necessary.
21. Pathogenesis.

In commencing the study of bacteriology the pupil should try the common staining methods and make the most important culture-media. Having culture-media prepared, it is customary to study a number of species of non-pathogenic bacteria. Notes of the work and sketches showing the morphology of the organisms should be made. In this as in other work with the microscope, the value of even crude drawings is very great as a matter of training. It is well to choose species which have properties decidedly different from one another. The micrococci, bacilli and spirilla should be represented; forms that are motile and that are not; species that form spores and others that do not form spores; some that liquefy gelatin and some that do not. There should be chromogenic forms , and species that ferment dextrose, and that produce indol,—such species as some of the sarcinæ, the bacillus coli communis, the hay bacillus, the potato bacillus, bacillus prodigiosus, a bacillus fluorescens and spirillum rubrum. It is well, when possible, to obtain material directly from nature rather than from laboratory cultures. This may readily be done in the case of the hay bacillus and the potato bacillus. Fecal matter may be spread on gelatin plates and the bacillus coli communis obtained in pure culture. Fluorescing bacilli are very common in water. Large spirilla are often found in swamp water. Some organisms like spirillum rubrum can only be had from laboratory cultures. An instructive experiment which anyone may carry out is to boil a potato thoroughly, and cut it into slices, placing these on moist filter-paper on glass plates, or on saucers, and after exposing them to the air for half an hour or more to cover them each with an inverted tumbler. Some of the slices prepared in this way should be put in the incubator, others left at room temperature. In a shorter or longer time there usually develops a great variety of isolated colonies from the bacteria that have fallen on the slices of potato. The growth of some aerobic organism, like the potato bacillus, may be tested under a cover-glass (see Fig. 29). The pyogenic bacteria, which can easily be isolated from pus, may be studied in this connection with great advantage. The staphylococcus pyogenes aureus and the streptococcus pyogenes should on no account be omitted. The diplococcus of pneumonia can most readily be obtained from a mouse or a rabbit which has died with pneumococcus infection. Such an animal can best be infected by subcutaneous inoculation, using some of the rusty sputum of a case of lobar pneumonia. The cultivation of the pneumococcus will be found to present difficulties in classes containing large numbers of students.

Representative forms of moulds and yeasts should be studied at the same time. Moulds are easily obtained by exposing some nutrient substance to the air, covering it, and allowing cultures to develop; yeasts will probably grow also. Ordinary brewer's yeast may be isolated in pure culture from gelatin plates. Bacteriological examinations also should be made of air, soil, water and milk. With such simple means, many of the important properties of bacteria may be demonstrated. It is most important that medical students should convince themselves by experiment of the extent to which bacteria are disseminated in our environments. The bearings of such observations on the practice of surgery and hygiene are obvious.

Experiments in sterilization and disinfection as described in Chapter VIII., Part II., may be performed with the bacteria mentioned, which present every variety of resisting power up to the almost incredible resistance of the spores of the hay and potato bacilli. The efficiency of the methods used for sterilizing surgical materials, as silk and catgut (Chapter IX., Part II.), should be tested; also, of the methods for disinfecting the hands, if possible, of the methods for disinfecting rooms, as well.

After some proficiency has been acquired, various pathogenic bacteria may be studied as the circumstances of the case require. Much judgment has to be used in allowing students to work with pathogenic bacteria. Anthrax, glanders, tetanus, cholera, bubonic plague, Malta fever, and diphtheria all have occurred in laboratory workers through accidental infection, sometimes with fatal results. Everything should be handled with forceps as far as possible, and the forceps sterilized in the flame before and after using. Particles of cotton fiber should not be allowed to fly off from the plugs. The various rules for the management of the platinum-wire, hanging-drop slides and sputum bottles, and for the handling of cultures and other infectious materials have already been given (pages 22, 23, 34 and 78 to 82). As the risks of infection from neglect of proper caution are obvious enough, it would seem, that it should be superfluous to warn students of the danger to themselves and others of infecting their hands and surroundings; but some who work in bacteriological laborities become careless, just as do those who work with explosives. The most important precaution, perhaps, is observance of the rule that while working in the laboratory, nothing should be put in the mouth. Cultures should never be left in improper places. Cultures of bacteria should be thoroughly sterilized before the tubes are cleaned. In some laboratories tubes, dishes and other apparatus, after use, are placed in the autoclave or in the dry sterilizer or they are soaked in disinfecting solutions; there seems to be no uniform practice in this respect.

In taking these measures, the same kind of reasoning applies as that which induces engineers to give bridges several times the strength they need to bear the greatest strain likely to be put upon them, or to make the boiler of a steam engine strong enough to bear six times the greatest pressure which it is expected that the steam contained in it will exert.

PART II.

CHAPTER I.

CLASSIFICATION; GENERAL MORPHOLOGY AND PHYSIOLOGY OF BACTERIA.

It has been universally agreed to class the bacteria as plants although they show resemblances to both plants and animals. On the one hand, they seem allied to the algæ and fungi, and, on the other, to the protozoa.

The classification of the larger animals and plants is based chiefly upon their morphology. With the bacteria this method of classification is applicable only to a limited extent owing to their extreme minuteness. And in addition it is necessary to resort to the grouping of the individuals as seen under the microscope. The difficulty in classification extends also to the matter of distinguishing one species of bacterium from another. Where no constant difference in morphology exists, resort is made to the grouping of the individuals as seen under the microscope, the presence or absence of independent motion, the naked-eye appearance of the growth upon culture-media and their physiological properties in relation to growth under various conditions of temperature, nutrition and relation to free oxygen. The agglutination of a species of bacterium by blood-serum specific for the species (see Chapter VII., Part II.) has also been used for purposes of identification.

These means of differentiation are not entirely satisfactory, and it is likely that forms which are now considered as different species are not really such in all cases. Notwithstanding the unsatisfactory condition of the classification of bacteria, it must not be supposed that the species of bacteria are not permanent. For instance, it would be incorrect to imagine that the micrococci and spirilla become converted into species of bacilli, or for the bacilli of one species to be transmuted into those of another. This is not in conflict with the statement that we may frequently, through erroneous and imperfect information, be in the habit of including unlike species under one name, or of classifying mere varieties of one species as entirely different species. At present it is sufficient for practical purposes to divide bacteria into two great groups—the lower bacteria and the higher bacteria; and to subdivide the lower bacteria into: *micrococci*, spherical forms; *bacilli*, rod-shaped forms, one diameter being in excess of the others; *spirilla*, twisted like a corkscrew, making long spirals or simply parts of spirals (comma-shaped forms).*

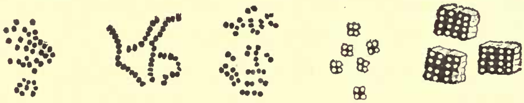
Staphylococci. Streptococci. Diplococci. Tetrads. Sarcinæ.

FIG. 41.

Recent investigations indicate that several species of bacteria often are closely related to one another, so as to form a well-marked group. Such a group is constituted by the bacillus of typhoid fever, bacillus coli communis and similar forms. The spirillum of cholera and other comma-shaped spirilla resembling it may be held to constitute another group. Still another is that containing the tubercle bacillus, smegma bacillus and other acid-proof bacilli.

The micrococci are subdivided into *staphylococci*, where the spheres grow in clusters like a bunch of grapes; *streptococci*, where they are arranged in long rows or chains, like a string of beads; *diplococci*, or pairs of micrococci; *tetrads*, where the individual spheres are grouped in fours; *sarcinæ*, where they are grouped in eights making the outline of a cube, resembling a bale or package tied with rope.

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*Migula's system of classifying bacteria has found favor with many writers.

The bacilli are not subdivided in this manner, although their forms vary considerably. The ends are sometimes square, sometimes round. Sometimes they are very short. Sometimes they grow in longer, thread-like forms, in which, however, the transverse markings which indicate the outlines of the individual bacilli can generally be seen, and which resemble a bamboo rod. Short, oval bacilli may look exceedingly like micrococci. Bacilli with rounded extremities, placed end to end, look like strings of sausauges. Under exceptional circumstances, branching forms of the bacilli of diphtheria, tuberculosis, glanders and bubonic plague and various other species have been encountered.*

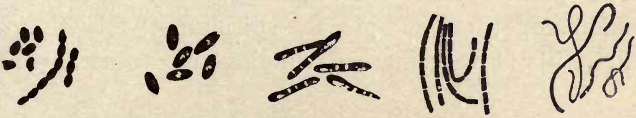

FIG. 32.—Bacilli of various forms.

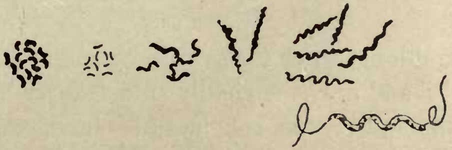

FIG. 43.—Spirilla of various forms.

The word "*bacterium*" was formerly used to designate short bacilli which generally formed no spores, while the word "*bacillus*" was restricted to the longer forms in which spore formation

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*See Hill. *Journal of Medical Research*. Vol. VII., January, 1902. Loeb. *Ibid.* Vol. VIII. 1902.

occurred. This use is no longer common, although not rarely the name bacterium is still given to a species—for instance, bacterium coli commune.

Spirilla present a very great variety of form. The short "*comma-shaped bacilli*" are only parts, at most, of spirals, although the microbes of cholera do sometimes form long spirals. On the other hand, there are among spirilla large and long sinuous figures which present most remarkable pictures under the microscope; for example, the spirillum of relapsing fever. Spirilla without very marked windings are sometimes called "*vibrios*"; and long, wavy forms with corkscrew-like windings "*spirochætæ*"; and only the rigidly spinal forms "*spirilla.*"

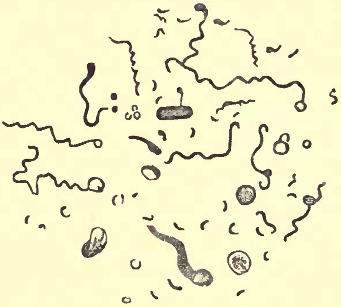

FIG. 44.—Involution forms of the spirillum of cholera.—(*Van Ermengem.*)

Besides the purely morphological classification already mentioned, bacteria are sometimes grouped according to certain physiological qualities. In general botany, saprophytes are plants that grow on decaying vegetable matter. In a bacteriological sense, *saprophytes* are bacteria which grow in external nature on dead organic matter, and *parasites* are bacteria which exist upon the living tissues or fluids of any organism. Nearly synonymous with the above words are those which do not and those which do produce disease, or *non-pathogenic* and *pathogenic*. The adjectives *facultative*, or optional, and *obligate*, or strict, are used to qualify the above terms and many others.

Size.—Bacteria vary greatly in size. The micrococci are usually 1 μ or less in diameter. The short diameters of bacilli and spirilla also are less than 1 μ, as a rule, while the length may be several micra. The anthrax bacillus (1.5 μ × 3 to 10 μ) and the spirillum of relapsing fever are the largest bacteria known to be pathogenic to man. To say that a micrococcus is 1 μ in diameter means that 25,000 end to end would make a line 1 inch long. It has been estimated that 1 milligram of a pure culture of the staphylococcus pyogenes aureus contains 8,000,000,000 micrococci.

There is good reason for believing that organisms exist which are too small to be visible with the most powerful microscopes. The nature of these organisms is not known, but it is not improbable that some of them are bacteria. (See pleuropneumonia of cattle etc., Part II., Chapter V.)

In stained preparations the bodies of bacteria frequently seem to be homogeneous. On the other hand, they may exhibit certain spots which stain more intensely than others, the stained spots alternating with clear areas. The dark-staining granules may take a slightly different shade of color from the rest (metachromatic granules, Babes-Ernst bodies). Somewhat similar appearances may result from changes in the density of the protoplasm of bacteria, leaving vacuoles that do not stain (plasmolysis).

In old cultures bacteria are likely to show irregular and often bizarre shapes, and these are called *involution forms*. It is not uncommon for bacteria to be enclosed in a kind of envelope of some clear substance, which stains with difficulty or not at all, called a *capsule*. The paired micrococci of pneumonia are enclosed in such capsules. The capsule is more likely to be demonstrated when the bacteria are obtained from the fluids derived from an animal's body than when they have been grown artificially in culture-media. A *zoöglœa* is a large mass of bacteria in a resting condition held together by a mucilaginous substance. The composition of bacteria varies considerably with different species. The basis appears to be proteid substance.

Vegetative Cells.—All the forms enumerated above are called *vegetative cells* in contradistinction to spores to be described later, and multiplication takes place by the direct division or *fission* of these cells. In the rod-shaped bacteria the fission is transverse. The formation of tetrads or sarcinæ from micrococci depends upon fission in two or three planes. Repeated fissions of microccocci in one plane result in the formation of streptococci. Micrococci that have recently divided are likely to be somewhat flattened on their opposing surfaces. Multiplication under favorable circumstances may take place at an exceedingly rapid rate. Bacilli have been observed to divide in twenty minutes. If division takes place once in an hour, the progeny of one organism at the end of twenty-four hours will be 16,777,216, *i. e.*, $(2 \times 1)^{24}$. The ordinary form of reproduction by fission is called *vegetative*, and bacteria that are multiplying in this manner are often spoken of as being in the vegetative condition.

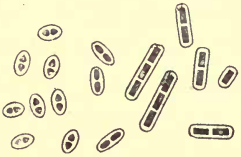

FIG. 45.—Bacteria with capsules.

Spores.—Under certain circumstances the reproduction of bacteria takes place by means of the germination of bodies called *spores*. These appear in a typical form in the large bacilli, where, near the centers of the bacilli, highly refracting, shining spots may be seen which are found to stain less readily with the aniline dyes than the rest of the bacilli. They are not to be confused with the unstained spots described as vacuoles. On account of their being formed from a part of the interior of the bacterium, such spores are called *endogenous*. These spores are found mostly in the bacilli, rarely in spirilla. They are what is meant when the word spore is used alone without qualification. The existence of another kind of spore, described as forming from the whole of the bacterium (called *arthrospore*), is doubtful. At all events, its significance is not at present understood. Spores develop generally, though not always, under adverse conditions of various kinds, as of temperature and of nutrition. They are more resistant to unfavorable influences of all sorts than are the fully developed bacteria. Spores, as a rule, resist drying, light, heat and chemical agents to a remarkable degree, but the spores from one and the same organism often differ in resisting power in different cultures. Frankland* found that anthrax spores which formed at 18–20° C. much more resistant than anthrax spores formed at 35–38° C. Even individual spores in the same culture differ in resisting power.

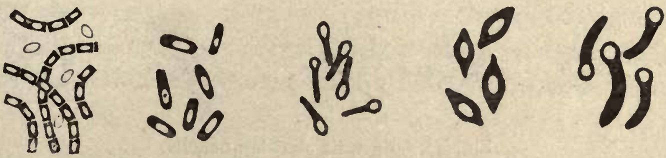

FIG. 46.—Bacteria with spores.

Anthrax spores are said to have been found which could withstand steam for twelve minutes, 1–1000 mercuric chloride for nearly three days, or 5 per cent. carbolic acid for more than forty days.† The greatest resistance is displayed by the spores of some of the saprophytic bacteria, particularly those of hay and potato, which are sometimes not destroyed by several hours of steaming; and bacteria which resisted 100° C. for sixteen hours are said to bave been obtained from the soil. When

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*Frankland. *Centralblat fr Bakteriologie*,etc. Bd. XV., 1894, p. 110.

†Günther. Einführung in das Studium der Bakteriologie. Leipzig, 1906, p. 406.

cultivated at a temperature as high as 42° C. for a sufficiently long time, the anthrax bacillus becomes incapable of forming spores; but as long as it retains any virulence at all it remains capable of forming spores.* Spores themselves do not multiply, nor do they manifest any activity. They may be located at the center of the bacillus or nearly at one end; in the latter case the end of the bacillus is likely to enlarge, making a form having the shape of a drumstick; this is seen in tetanus bacilli (Fig. 44). When a bacillus assumes a spindle shape on account of having the middle part bulged through the formation of a spore it is called a *clostridium*. With rare exceptions, a single bacillus contains but one spore. Under favorable conditions the spores germinate, as it is called, and develop to the adult form of the organism. This may be witnessed in hanging-drop preparations.

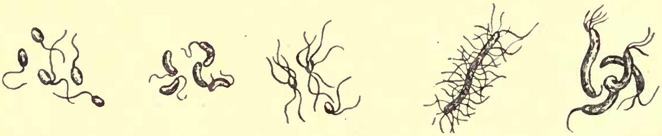

FIG. 47.—Bacteria showing flagella.

Spore formation is not a method of multiplication, since one spore when it germinates reproduces but one cell, and this cell then multiplies. So spore formation seems to be a means of preserving the organism under unfavorable environments, and is not a process of reproduction in a strict sense.

Motility.—Motility is rarely exhibited by micrococci; some bacilli possess it and some do not; while nearly all of the spirilla are motile. The phenomenon is observed in the hanging-drop. The degree of motility is variable, being sometimes slight and sometimes very active. When seen under a high power the little particles taken from a culture of a motile

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*Kolle and Wassermann. *loc. cit.* p. 42.

organism may look like a writhing mass of maggots or like tadpoles in a pool. The motility is most active in young cultures. The movement results from the vibration of little processes, or *flagella* (Fig. 45). Of these there may be one or several, placed singly or in groups, at the end, or scattered around the sides. They are extremely difficult to demonstrate except by special staining methods, which, furthermore, are quite uncertain of result. After the flagella have been stained, the bacteria appear somewhat larger than when stained by the ordinary methods. The flagella upon the bacilli of typhoid fever are numerous and form a very striking picture.

Chemotaxis.—Motile bacteria possess the property of being attracted by certain substances (positive chemotaxis) and of being repelled by others (negative chemotaxis). Similar properties are widely distributed among living cells, both animal and vegetable.

FAVORABLE AND UNFAVORABLE CONDITIONS FOR GROWTH.

Warmth.—Among the different kinds of bacteria forms exist which multiply at temperatures as low as 0° C., while there are species that multiply at 70° C. Bacteria which flourish at a very high temperature (maximum about 70° C.) are called *thermophilic.* The pathogenic bacteria usually flourish better at a point somewhere near the temperature of the human body. This is not necessarily the case with the non-pathogenic species. Ordinary water bacteria thrive best at ordinary temperatures.

Sternberg's method for determining the *thermal death-point* of a species of bacteria is to draw a portion of a pure culture of the organism to be tested up into a capillary tube which has a small bulb on one end, and after sealing the end of the capillary tube in the flame the tubes are placed upon a glass plate in a water-bath, whose temperature is indicated by a thermometer, while a uniform temperature is secured by stirring.

The time of exposure is, as a rule, ten minutes. The tubes should be removed quickly to cold water. Their contents should afterward be inoculated into bouillon to determine whether or not the organisms have been killed. In the practical use of heat for sterilization or disinfection, the exact thermal death-point is greatly exceeded. The time of exposure is also longer than is absolutely necessary as determined by the results of the experiments.

It is hardly safe to depend upon text-book statements in regard to the thermal death-point of bacteria in practical disinfection.

Moisture is indispensable to the growth of bacteria, and drying causes the death of certain kinds, as, for instance, the spirillum of cholera, while others remain alive, but do not grow.

Heim* found that the resistance of organisms to drying is very much greater when the organism in question is contained in the pathological material from animals which have succumbed to the disease, on the one hand, than when it is derived from cultures, on the other. The pneumococcus which is very sensitive to drying, and in fact is difficult to keep going on culture media, remains alive for 16 months and preserves its virulence for more than a year when it is contained in blood from an animal which has died of the infection dried on silk threads and kept in a desiccator containing calcium chloride. Similar results were obtained with other organisms.

Food.—There are a few species of bacteria that contain chlorophyll, but it is wanting in most forms. On account of the absence of chlorophyll, bacteria require, as part of their food, organic compounds, such as sugar, as a source of carbon. They are unable, with very few exceptions, such as the nitrifying bacteria, to derive their carbon from the carbon dioxide of the atmosphere, or from inorganic carbon compounds. Although some species are able to obtain nitrogen from inorganic salts, most bacteria flourish best if organic substances containing nitrogen, like peptone and albumen, are furnished them as part of their food. The complicated, unstable, organic molecules with high potential energy are converted by them into simple and more stable compounds like carbon dioxide, ammonia and water, with the liberation of energy. These facts become manifest in connection with their important work in decomposition, putrefaction and fermentation. A culture-medium having a slightly alkaline or neutral reaction is favorable to most bacteria.

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* *Zeitschr. f. Hygiene.* Bd. L., No. 1, April 4, 1905, p. 123.

The prolonged artificial cultivation of bacteria may or may not modify their properties. The pathogenic bacteria are likely to undergo considerable modification both in the quality and luxuriance of their growth and the intensity of their pathogenic characters.

The growth of bacteria may eventually be hindered by the accumulation of the products of their own metabolism. Many bacteria refuse to grow on culture-media at all; at least the suitable artificial medium has not yet been found for them. Some species are extremely fastidious, and can only be propagated on particular nutrient substances, others again will grow in distilled water.* But bacteria show great adaptability, and, once they have been made to start, they can be further cultivated with less and less difficulty, as a rule.

Relation to Oxygen.—Oxygen is indispensable to the growth of bacteria. Some of them, the *aerobes*, require oxygen in the free form. Others, the *anaerobes* require it in some form of compound, and are unable to live in an atmosphere of free oxygen. Others still are able to flourish either in the presence or absence of free oxygen-facultative aerobes or anaerobes. The first-named varieties are sometimes called strict, or obligate aerobes or anaerobes.

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*Bolton. *Zeitschrift für Hygiene.* Bd. I., No. 1, 1886.

CHAPTER II.

PRODUCTS OF THE GROWTH OF BACTERIA.

THE splitting up of animal and vegetable matter by the bacteria results in the formation of various products, which may possess certain characteristics. Thus some of the products of bacterial growth are phosphorescent, some are marked by more or less vivid color, others again by poisonous properties.

Phosphorescence.—Bacteria whose cultures exhibit phosphorescence have been found in the ocean and in fish.

Chromogenic Bacteria.—Many bacterial growths display brilliant coloring. The different species of sarcinæ are remarkable for forming highly-colored growths; some of them are rose-red, some orange-yellow, some lemon-yellow, and so on. The bacillus prodigiosus presents a brilliant red growth whose rapid development is said to have formed the basis for the so-called "Miracle of the Bleeding Host" (see page 5). The bacillus pyocyaneus in culture gives a brilliant green fluorescence and is responsible for the color of blue or green pus. Bacilli which exhibit a green fluorescence in cultures are common in water. In cultures on potato or agar the colors of the chromogenic forms are usually well shown. The pigment formed by the chromogenic bacteria is not produced in the cells themselves. These are colorless. The color is due to substances excreted by the cells or formed from material in the culture-media.

Ferments or Enzymes.*—Many bacteria form ferments which have the power of dissolving proteid substances in a manner similar to trypsin. The liquefaction of gelatin is a

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*Consult Buxton. Mycotic Anzymes. *American Medicine.* July 25, 1903.

familiar example of this process. The property of liquefying gelatin, or the failure to do so, is used in classifying bacteria and in determining the nature of unknown species.

Some bacteria, as the bacillus coli communis, form ferments which act like rennet in coagulating milk. Other bacteria are capable of forming sugar from starch. Others have the power of changing cane -sugar into glucose.

Bacteria which are able to decompose cellulose are found in the stomachs of ruminant animals. Although it is doubtful whether the products of cellulose decomposition have any nutritive value, the process is probably useful in effecting a subdivision of the coarse food, consisting of grass, hay and the like.

Some bacteria have the power of decomposing neutral fats into fatty acids and glycerin, after the manner of the fat-splitting ferment of the pancreatic juice.

The *end-products* which result from the growth of bacteria upon albuminous nutrient media are very numerous. They are complicated and not well understood. Among these end-products may be mentioned peptone, indol, skatol, phenol, leucin and tyrosin. Nearly related are the toxins (see Chapter VI.), which play an important part in the production of disease by pathogenic bacteria. In the decomposition of urine by bacteria the urea is converted into ammonium carbonate.

The *formation of indol* in cultures is an important peculiarity of certain bacteria. The manner of making the test for indol is somewhat differently described by different authors, but the Committee on Standard Methods of Water Analysis, American Public Health Association,* recommend the following:

The organism to be tested must be made in broth from which all traces of muscle sugar have been removed or in peptone broth. The cultures must be incubated at 37° C. for four days. In applying the test two drops of concentrated

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**Journal of Infectious Diseases.* Supplement No. 1, May, 1905, p. 115. See also in this connection Tobey. *Journ. Med. Research.* XV., 1906, p. 301.

sulphuric acid and one c.c. of a 0.01 per cent. solution of sodium nitrite are added to the culture after it has been allowed to grow for this length of time, and after this addition the culture is observed at the end of one-half hour. The appearance of a pink color indicates the presence of indol.

Another method, and quite a delicate one, after adding the sodium nitrite, is to run in a layer of sulphuric acid beneath the culture so as to form a layer of acid below and culture above. The presence of indol is indicated by a pink ring at the point of juncture of the acid and the culture. If the reaction is obtained with sulphuric acid alone without the addition of the nitrite, it indicates that both nitrites and indol are present in the culture. Blank tests must be made with the same culture-medium which is employed for the cultures, since, as was shown by Wherry,* nitrates and probably also nitrites may gain entrance to artificial culture, media from various sources, such as the water, peptone and filter-paper used in preparing the media. Wherry also showed that a sufficient quantity of nitrites may be absorbed from the air of the laboratory in a few days to yield a distinct indol reaction.

Rivas † found that a much more delicate test than the above consists in the use of 1 c.c. of a 10 per cent. solution of sodium hydroxide in place of the sodium nitrite, and the use of 1 c.c. of 50 per cent. sulphuric acid. These are mixed with the culture to be tested. This test gives a bright purple or pinkish coloration with outlines of B. coli, but not with its congeners.

Check blank tests are especially demanded where the culture-medium has been prepared by growing Bacillus coli in the meat infusion to free it from sugar, since Bacillus coli itself forms indol in appreciable amounts.

The Committee on Methods of Identification of Bacterial

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*Wherry. *Journal of Infectious Diseases.* Vol. 2, 1905, pp. 436–445.
†*Journ. Infec. Dis.* Vol. IV., No. 4, Nov. 15, 1907. pp. 642–646.

Species, Society of American Bacteriologists, recommends that ammonia and indol tests be made on cultures at the end of the tenth day, nitrite tests at end of the fifth day (see Appendix I., Note 5).

The reaction may be hastened by warming slightly. The value of this reaction will be understood when, to give one illustration, it is remembered that the bacillus coli communis usually, if not always, produces indol and the bacillus of typhoid fever usually does not, except in cultures kept for some time—ten days or more. Morris* found that in cultures of B. typhosus, cultivated at 37 ° C. for this length of time, indol was always present, but not in cultures of this organism which were one or two days old. The recommendation of the Society of American Bacteriologists that the indol test should be made in cultures ten days old would seem not to apply for differentiation of B. typhosus. The reaction depends upon the liberation of nitrous acid which, with indol, forms a red color.

The change of organic substances into more stable ones does not cease with the compounds mentioned above. Certain bacteria of the soil, which will be discussed further on, are able to complete the conversion of ammonia into nitrous acid, leading to the formation of nitrites; and others still that of nitrites into nitric acid, which at once forms nitrates.

Formation of Acids.—In the course of their growth many bacteria produce acids, especially from substances containing sugar. The power of developing lactic acid is possessed by a large number of species. Acetic acid is another common by-product. Besides these, butyric acid, formic acid, propionic acid and many more are formed by different bacteria.

Development of Gas.—The evolution of gas from bacterial growths is of frequent occurrence. Many bacteria have the property of splitting up organic compounds with the formation of various gaseous products. Perkins* has found that the power of thus breaking up sugars with the formation of gaseous products may be lost in whole or in part by modification of environment. In some cases the power would seem to be permanently lost, in others it may be recovered again. This observation makes it necessary to interpret with great caution the results of fermentation tests as a specific means of differentiation between organisms which are otherwise alike. It is important to bear this fact in mind particularly in the diagnosis of the members of the colon group. Carbon dioxide, hydrogen sulphide and nitrogen are among the better known gases that may be formed. The odors that arise from cultures and that are so characteristic of putrefactive processes depend upon the development of gases, or a mixture of gases, of considerable complexity. The bacillus aerogenes capsulatus leads sometimes to the formation of gas in the organs of the human cadaver within a short time after death. Theobald Smith introduced a valuable means of differentiating species of bacteria based upon their power of forming gas in media containing different sugars, or in their inability to do so. Bouillon containing 1 per cent. of dextrose (or lactose, etc.) is the culture-medium advised. The test is best conducted in a **U**-shaped tube, closed at one end, and at the other end provided with a bulb (Fig. 48). The tube is stoppered with cotton, sterilized

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*Cited from Günther. Einführung in das Studium der Bakteriologie. 6te Auf., 1906, p. 526.

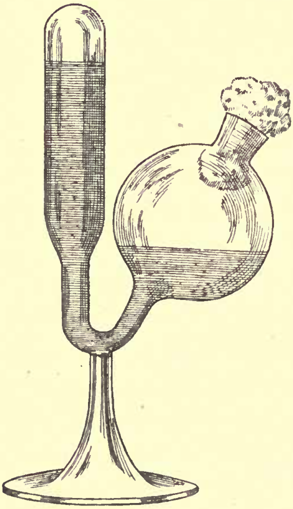

FIG. 48.—Fermentation-tube.

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*Perkins. *Journ. Infect. Dis.* Vol. 4 No. 1, pp. 51–65.

by dry heat, afterward filled with the bouillon, and sterilized by steam in the usual manner. After the last sterilization it should be tilted until the closed end is completely filled with the medium. After it has been inoculated with the species under consideration, any development of gas will be indicated by the collection of the gas at the closed end. The amount of gas formed may be estimated and its quality tested. To accomplish the latter fill the bulb with 2 per cent. solution of sodium hydroxide, close the outlet, and tilt the tube to allow the mixture to come in contact with the gas. After shaking, this causes the absorption of the carbon dioxide and diminution in the quantity of gas. The portions which remain may be mixed with air and ignited, when the presence of hydrogen and some of its compounds will be indicated by an explosion. (See The Detection of Bacillus coli communis in Water, Part IV.)

The development of gas may readily be tested by inoculating the bacteria by a deep puncture into agar containing 1 per cent. of dextrose or other sugars. The development of gas causes bubbles to form in the agar, often to the extent of splitting it, and sometimes forcing out the cotton plug (see Fig. 73).

The activities of bacteria which have just been enumerated are fundamental to the phenomena which go by the names of *fermentation* and *putrefaction*. These words have been defined differently at different times and by different writers, but in general both are used as names for the breaking up of complex organic compounds by micro-organisms with the formation of simpler compounds. Fermentation refers especially to the formation of useful products like alcohol. The term putrefaction is employed chiefly for the breaking up of nitrogenous compounds with the development of foul-smelling gases. The term fermentation is also applied to the decomposition of complex substances through the influence of unorganized ferments or enzymes. The work of bacteria in decomposition is indispensable to the existence of the organic world as we find it.

Green plants convert the stable compounds of nitrogen, the carbon dioxide of the atmosphere and water into the complex and unstable albumens and carbohydrates which serve as food for animals. Animals, on the other hand, convert these unstable and complex compounds back into simpler forms. The work of changing them back into the simple and stable condition, in which they serve as the food for plants, is performed by animal life in part only, and its completion is left to the activities of bacteria. It is the work of bacteria in this direction which we call decomposition. Without that work the existence of life upon the earth, as we understand it, would soon come to an end, and the dead and undecomposed bodies of living things and their products of all kinds would lie about unchanged, as they had fallen.

Bacterium termo is the name formerly given to a supposed species of bacteria which was credited with being the producer of putrefaction. The individuals were represented as being short rods, mostly growing in pairs, and actively motile. The term has been abandoned since it appears to have included a number of different species.

CHAPTER III.

THE BACTERIA OF SOIL, AIR, WATER, AND OF MILK AND OTHER FOODS.

The Bacteria of the Soil.—Bacteria are present in the soil in enormous numbers—100,000 or more in 1 c.c. of virgin soil, according to Flügge. The depths to which they penetrate depend upon the character of the soil and the character of the life upon it, and whether or not it has been artificially disturbed, as by cultivation. In general, at a depth of 1.25 meters (about four feet) the number becomes very small, and at a depth of a few decimeters more the soil may be found entirely sterile.*

The bacilli of tetanus and malignant edema, and bacillus aërogenes capsulatus are present in the soil of many localities. According to Woodhead, certain savage tribes of Africa and the East Indies use as an arrow-poison soil that is capable of producing tetanus. The bacillus of anthrax may be found in soil which has been infected with this organism.

Most of the bacteria of the soil are harmless and some of them are useful saprophytes.† To the latter class belong the *nitrifying bacteria* described by Winogradsky and by Jordan and Richards and those organisms occurring in soil which have the power of converting ammonia into nitrous acid which forms nitrites, and others which complete the change of nitrites into nitrates. Both kinds are widely distributed. These organisms will not grow on ordinary culture-media, and their cultivation presents great difficulties. Probably a good many bacteria have similar properties to some extent. The work done by nitrifying bacteria in the formation of nitrates from sewage, manure and the like is indispensable to most plant life. Certain bacteria found in the soil are also concerned in the assimilation of free, atmosphere nitrogen, resulting in the addition of a valuable proportion of nitrogen compounds to the soil. This is spoken of as *nitrogen fixation.* Inasmuch as a large part of the excrementitious products of animals containing nitrogen are not retained in the soil, where they may be employed as food by plants, but are washed directly or indirectly into the sea by means of sewage and the rivers, it will be seen that the supply of nitrogen compounds might suffer gradual exhaustion. Furthermore, it has already been noticed (page 131) that one of the products of decomposition by bacteria is nitrogen, which is not available as food for animals or for most plants. These facts have met with practical recognition by agriculturists in the adoption of various methods of fertilizing the soil. It appears that the roots of peas, beans, clover, alfalfa and some other plants frequently present minute tubercles which are caused by the development of microörganisms related to the bacteria. These organisms appear to have the power of assimilating atmospheric nitrogen and of converting it into nitrogen compounds. The same property probably belongs to some other bacteria of the soil. Experiments show that these observations may be destined to be of great value to the farmer.*

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* Güther, *Soc. cit.* p. 291. See also Voorhees and Lipman. W. S. Dept. of Agr.Exp. Stat. Bulletin 194. Oct. 26, 1907.

† See Conn. Agricultural Bacteriology.

The bacteria of the soil may easily be studied in plate-cultures made from small portions of soil collected with the necessary precautions to avoid contamination, or plate-cultures may be made from sterilized water with which a portion of the soil has been properly mixed. Anaerobic bacteria must be cultivated by the special methods adapted to them.

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*For practical application to agriculture consult G. Moore. U. S. Dept. Agriculture. Bureau of Plant Industry, Bulletin No. 71, Jan. 23, 1905. See also various Bulletins of the Department of Agriculture on this subject.

Bacteria of the Air.—The bacteria of the air will be found for the most part clinging to solid particles in suspension in the shape of dust also, as shown by Flügge, to particles of sputum thrown out by efforts in coughing. The minute air bubbles thrown off in this way remain suspended for some time. Still, as has already been stated, bacteria cannot be blown from moist surfaces, they are not removed by currents of air. Conditions of dryness and wind tend to increase the number of microörganisms in the air. They are fewer after a fall of rain or snow, and the number is smaller in winter than in summer. The air of cities contains more bacteria than that of the country. The atmosphere over the sea and at the tops of high mountains is nearly or wholly free from bacteria. The bacteria which do occur in the air are seldom pathogenic. Their character depends upon the character of the dust. It is obvious that dust which consists in part of the dried, pulverized expectoration of cases of pulmonary tuberculosis may contain tubercle bacilli. Anthrax of the lungs sometimes arise in men who handle the wool of sheep that were infected with anthrax (wool-sorter's disease), and is due to the inhalation of anthrax spores attached to the wool. The atmosphere in the immediate vicinity of cases of the exanthematous fevers is liable to contain the organisms, whatever they may be, that cause these diseases.

In a rough way, one may obtain some knowledge of the character of the oranisms in the air of a given locality by removing the cover of a Petri dish containing sterilized gelatin or agar or thin slices of boiled potato for a few minutes, replacing it, and allowing the organisms to develop. In most cases a large proportion of the growth that appears will be moulds. Yeasts are also common, and among the bacteria the micrococci are abundant. Chromogenic varieties are likely to be present.

A few studies of this character will show that the number of organisms that are present depends chiefly upon whether the air is quiet or has recently been disturbed by draughts, gusts of wind or sweeping. These facts are of fundamental importance in laboratory work, where plate-cultures are being studied, if we wish to avoid contamination of the plates. Among various devices that have been proposed for the accurate study of the organisms of the air, the Sedgwick-Tucker aërobioscope is the simplest and most accurate. It consists of a glass-cylinder (Fig. 49) a few inches long and an inch or two in caliber with a narrow neck at one end and a narrow tube annealed to the other. A layer of granulated sugar of an inch or more is packed loosely in the narrow tube, and the neck and the end of the narrow tube are plugged with cotton. The instrument is sterilized in the hot-air sterilizer. After removing the cotton a definite quantity of air is to be aspirated through the large end, which may be done by means of a suction-pump applied to the other end, or by siphoning water out of a bottle the upper part of which is connected with the end of the aerobioscope by means of a rubber tube. The sugar acts as a filter and sifts out of the air the microörganisms which are contained in it. Liquefied gelatin or agar in sufficient quantity is introduced into the large end of the instrument by means of a bent funnel; and, after replacing the cotton, it is mixed with the sugar which dissolves. The culture-medium is spread around the inside of the larger portion of the tube after the manner of an Esmarch roll-tube. The bacteria which are filtered out by the sugar develop as so many colonies upon the solidified medium.

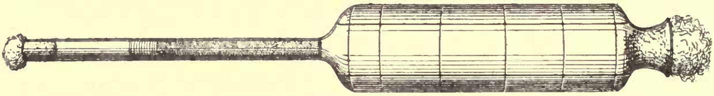

FIG. 49.—Sedgwick-Tucker aërobioscope.

Bacteria of Water and of Ice.—The water of rivers, lakes and the ocean always contains bacteria. The number of organisms varies greatly in different places and under different conditions. The number of different species found in water is also very large. Ground-water* contains few or no bacteria under normal conditions, and is therefore suitable for a source of water-supply, when a sufficient amount is available. The possibility of contamination of the ground-water from unusual or abnormal conditions should always be eliminated before it is taken for drinking-water. Numerous epidemics of typhoid fever have been traced to contamination of wells. The location of wells with reference to privy-vaults, and other possible sources of contamination should be chosen with the greatest care.

The ordinary bacteria of water† are harmless, as far as is known. Bad ordors and tastes in drinking water that is not polluted with putrid material are usually due to minute green plants (algæ).‡ The diseases most commonly disseminated by water are typhoid fever and Asiatic cholera, and probably also dysentery. The results of experiments testing the length of time which the cholera spirillum and the typhoid bacillus may persist in water are conflicting. Many epidemics of cholera and typhoid have been traced to water polluted with the discharges from cases of these diseases.

By *self-purification* of water is meant the removal through natural processes of contaminating organisms such as might occur from the discharge of sewage into it. It depends upon the sedimentation of the contaminating material, in the form of mud, upon the growth of the ordinary water-plants and protozoa, upon the exhaustion of the food-supply by the growth of bacteria themselves, upon the destructive influence of direct sunlight, and the dilution of the matter added with a large volume of water.* It is not usually to be relied upon as a means of freeing the water-supply from pathogenic bacteria.

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*Ground-water is the water which—originally derived from rain or snow—sinks through superficial porous strata, like gravel, and collects on some underlying, impervious bed of clay or rock.

†See Fuller and Johnson The Classification of Water Bacteria. *Journal of Experimental Medicine.* Vol. IV., p. 609. Jordan. *Journal of Hygiene.* Vol. III., Jan., 1903.

‡G. T. Moore. Contamination of Water Supplies by Algæ. Yearbook U. S. Department of Agriculture. 1902.

Storage of Water.—When water is kept in large reservoirs, the solid particles in it, including bacteria, tend to fall to the bottom. The number of bacteria in a water-supply may be considerably reduced in this way by allowing sedimentation to take place and using the upper portion of the water.

Filtration.—Filtration on a large scale has been more commonly in use in the cities of Europe than elsewhere until lately. But filtration-plants now exist in several cities of the United States. By this method 98 per cent. to 99 per cent. of the bacteria in water may be removed.

Slow Sand Filtration.†—The filter consists of successive layers of stones, coarse and fine gravel. The uppermost layers are of fine sand. The whole filter is from 1 to 2 meters thick. The sand should be 60 cm. to 1.2 meters in thickness. The accumulated deposit from the water and a little of the fine sand must be removed from time to time, but the layer of fine sand must never be allowed to become less than 30 cm. in thickness. The first water coming from the filter is discarded. The actual filtration is done largely by the slimy sediment which collects on the surface of the layer of fine sand. The filter-beds may be several acres in extent, and in cold climates should be protected by arches of brick or stone. They require renewal occasionally. This kind of filtration has come largely into use since the cholera epidemic of 1892–93, and it appears to be very effective. It is often advisable to use

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*See Jordan. *Journal of Experimental Medicine.* Vol. V., p. 271.

†For a full discussion see *Journal American Medical Association.* Oct. 3, to 31, 1903.

storage basins in connection with sand filtration, to permit of settling of part of the solid matter before filtration.

The results obtained by filtration depend greatly upon the intelligence displayed in operation.

Mechanical Filtration.—This method of filtration is also called the American system. It is more rapid than the preceding method and does not require a large area for filter beds. Although sand is required also, filtration is accomplished by a jelly-like layer of aluminum hydroxide. This product is formed by adding to the water a small quantity of aluminum sulphate or of alum. The carbonates in the water decompose the aluminum salt and produce aluminum hydroxide. It precipitates as a white, flocculent deposit, entangling solid particles, including bacteria, as coffee is cleared with white of egg. Only a trace of aluminum should appear in the water. This method of filtration has not been tested so extensively as slow sand filtration, but seems likely to prove efficient. With water, poor in carbonates, these may have to be added.*

Whipple and Longley† found that the efficacy of mechanical filters with the addition of alum depends somewhat upon the character of the alum. They find that the alum shall be shown by analysis to contain 17 per cent. of alumina (A12 03) soluble in water, and of this amount at least 5 per cent. shall be in excess of the amount necessary theoretically to combine with the sulphuric acid present. It shall not contain more than 1 per cent of insoluble substances, and shall be free from extraneous debris of all kinds. It must not contain more than 0.5 per cent. of iron (Fe_2O_3) and the iron shall be preferably in the ferrous state.

Various methods for the purification of water by means of chemicals have been proposed. The use of copper sulphate to disinfect drinking water was recommended by Moore

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*See Fuller. *Journal American Medical Association.* Oct. 31, 1903

†*Journ. Infect. Diseases.* Supplement No. 2, Feb., 1906, pp. 166–171

and Kellerman,* and various investigators tested the value of their recommendation.

Clark and Gage† came to the conclusion from their investigation that the treatment of water with copper sulphate or the storing of water in copper vessels has little practical value. Others also have come to practically the same conclusion. While the addition of copper sulphate is of use in preventing the growth of the algæ, which sometimes grows so abundant, as to choke up water pipes, is of benefit in this direction, the weight of evidence appears to be against its efficacy for purifying water for drinking purposes. The use of ozone for the purification of water has met with considerable favor.‡

The filtration of water on a small scale, as is ordinarily done for domestic purposes, is generally entirely useless. The so-called Pasteur filter of unglazed porcelain is effective if it is properly constructed and if the filter-tubes are sterilized by heat every few days—conditions which are seldom complied with. Distillation of water and boiling are the most practical methods for sterilizing drinking-water.

Collection of Samples.—For bacteriological examination samples from the water-supply of a city may be drawn from the faucet, but the water should first be allowed to run for half an hour or longer. From other sources the supply should be collected in sterilized tubes or bottles, taking care to avoid contamination. Sternberg bulbs (see Fig. 38) will be found useful for small samples. These samples should be examined as quickly as possible, for the water bacteria increase rapidly in number after the samples have been collected. When transportation to some distance is unavoidable the samples should be packed in ice, but even this precaution does not preserve the original bacteriological condition of the water at the time

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*U. S .Dep. Agriculture. Bu. Plant Ind. Bulletin 64, 1904.
†*Journ. Inf. Diseases.* Sup. No. 2, Feb., 1906, pp. 175–204.
‡Consult Rosenau. Disinfection and Disinfectants. 1902.

it is caught; for more or less change probably takes place at all temperatures. If the temperature is too high, and the water freezes, more or less of the bacteria may be killed; if, on the contrary, the temperature is not low enough there will be a multiplication of the bacteria in transit. The value of examination of water shipped in any way is of, at least, doubtful utility.

The **number of bacteria** may be determined by making plates of a definite quantity of the water with gelatin or agar.* The amount examined ordinarily is 1 c.c. When the number of bacteria is very large, a smaller quantity must be taken, and it may be necessary to dilute the sample ten times or more with sterilized water. The amount should be measured with a sterilized, graduated pipette. The water is mixed with melted gelatin or agar in a tube which has been allowed to cool after melting. After thorough mixing, remove the plug, burn the edge of the tube in the flame, hold in a nearly horizontal position until cool and pour into a sterilized Petri dish. Or better, measure the water into the Petri dish, and pour the melted medium in, and mix. The number of colonies may be counted on the third or fourth day; the later the better, as some forms develop slowly and may not present visible colonies for several days; but the plates are often spoiled after three or four days by the profuse surface growths of certain forms or by the rapid liquefaction of gelatin, if that be used, by other forms. The number of colonies that develop is supposed to represent the number of individual bacteria contained in the quantity measured. That will probably not always be the case, however, as colonies may develop from a clump of bacteria which have not been separated from one another by the mixing process. Abbott has shown that the number of colonies is usually larger on gelatin plates than upon agar plates, and at the room temperature than in the incubator.

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*For preparation of culture media for water analysis, see p. 71.

This observation illustrates the fact that there are doubtless many kinds of bacteria that do not find favorable conditions for development on ordinary culture-media. The reaction of the medium has an important influence upon the development of these water bacteria in plate cultures.

When the number of colonies is small, there is no difficulty in counting them as they appear in the ordinary Petri dish. When the number is large, some kind of mechanical device may be used to assist counting. The Wolffhügel plate is a large square of glass resting in a wooden frame painted black. The glass plate is ruled in squares. It was designed particularly with reference to the form of plate-cultures first made by Koch. The Petri dish, however, may be placed upon the glass plate and the cross lines be used to assist in counting. Lafar, Pakes and Jeffer recommend a surface painted black, ruled with white lines which represent the radii of a circle, which may be still further subdivided by other lines. Many find counting easier when a black surface divided into squares is employed. An ordinary card with a smooth black surface divided into squares by white lines may be placed under a Petri dish and will be found to serve very well.* For the mere examination of the colonies no better surface can be devised than the ferrotype plate used by photographers. The examination of the colonies will be easier if a small hand-lens be used. Care must be taken not to mistake air-bubbles or particles of dirt for colonies of bacteria.

In any case, if possible, all the colonies in the plate should be counted. But if this is not possible, the number contained within several squares may be counted and the average taken; knowing the size of the squares and the area of the plate, the number contained in the whole plate may be calculated.

The plating may be done by rolling the medium after the manner of Esmarch. When the number of colonies is not large this may serve very well. Counting may be assisted by drawing lines with ink on the outer surface of the test-tube. It is obvious that the character of the bacteria is of prime importance; that pathogenic organisms may occasionally be present, even when the number of bacteria to the cubic centimeter is small. But knowing the number usually found in a good water-supply, any sudden variation above that number is to be looked upon with suspicion as indicating a possible contamination.

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*Specially ruled cards will be found after the Appendix II., at the back of the book.

The bacteriological examination should always be accompanied by a chemical examination, and by an inspection of the surroundings. A large number is to be expected when the water has been subjected to unusual agitation from winds or currents which stir up the bacteria which have settled.

The detection of pathogenic bacteria in water* involves great difficulties, and our knowledge in this direction is very meagre. Koch and several others have reported finding the spirillum of Asiatic cholera in water. The examination of water-supplies for this organism has disclosed the fact that bacteria resembling the organism of cholera in many respects are not uncommon in water. This adds to the difficulty of detecting the cholera germ in water.

The bacillus of typhoid fever has many times been described as occurring in water-supplies suspected of being contaminated with the excreta of cases of the disease. The interpretation of these observations is at present doubtful.† It is now known that several forms of bacteria exist which closely resemble the bacillus of typhoid fever, and which make its recognition in an unknown specimen very difficult.‡

It will at once be appreciated that the number of cholera and typhoid organisms necessary to contaminate a considerable body of water, and sufficient to cause an outbreak of the disease among some of the people drinking the water, may still be so small that many different cubic centimeters of the water might be studied before a single one of the specific organisms would be encountered. Anyone who has examined plates made from samples of water will recognize the difficulty of detecting one or a few colonies of the bacteria of cholera or typhoid fever among a hundred or more colonies of ordinary water-bacteria. The existence of contamination with animal excreta might, however, be indicated by finding the bacillus coli communis, whose detection offers a greater prospect of success, the presence of small numbers of the colon bacillus in water is regarded as of little or no significance.* Until our knowledge is more complete, any suspicious water should be discarded.

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*See also articles in Part IV. on the bacillus of typhoid fever, bacillus coli communis and spirillum of cholera.

†Consult editorial. *Journal American Medical Association.* Dec. 5, 1903.

‡For methods of detection see under Typhoid Bacillus, p. 370.

Formerly investigators seem to agree that if, using several samples of a water each 1 c.c. in volume, colon bacilli are found in a majority of the samples the water is probably polluted; if the colon bacillus is only found when larger volumes of water are examined, the results are suspicious though less significant. Some investigators hold that the presence of streptococci in water is indicative of pollution.† At present there seems less agreement upon these points. Johnson ‡ found that the colon bacillus is ingested by fish when this organism is present in the water in which the fish are kept, and that the bacillus lives and multiplies in the intestines of the fish. He concludes that in this way fish may convey the colon bacillus, and if so also the typhoid bacillus, from a contaminated source to an uncontaminated stream.

Certain devices have been adopted to hasten the development of the bacteria indicative of pollution and to retard that of the ordinary water-bacteria. Among these may be mentioned the influence of the heat of the incubator, which will hasten the growth of organisms derived from the human body, and which retards the growth of water-bacteria. Another

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*Jordan. *Jaurn. Am. Med. Assn.* V. XLVIII., No. 22. June 1, 1907, p. 1861.
†Prescott and Baker. *Journal of Infectious Diseases.* I. 193.
‡*Journ. Infect. Diseases.* Vol. I., pp. 348–354.

is the addition of a solution of peptone to a large quantity of the water to be examined with a view to assisting the development of the desired bacteria by furnishing them suitable food for growth. In another method (Parietti's) small quantities of carbolic acid are added to bouillon and mixed with the water, with a view to retarding the development of all except typhoid* and colon bacilli; Jackson† advocates the use of ox-bile for the same purpose. The committee on Standard Methods of Water Analysis, Am. pub. Health Assn.,‡ suggest the following modifications of previous recommendations for the detection of B. coli in water:

Omit determinations of motility: omit tests for coagulation of milk in case cultures have been isolated in lactose agar plates; omit determination of the amount of gas formed and the gas ratio in both presumptive and in confirmatory tests; allowing the use of bile-broth instead of dextrose-broth. Suspected bacteria may be tested by inoculation into animals; the possession of pathogenic properties is thought by some to create a probability in favor of their having come from some contamination with animal excreta.§

If it is not already apparent from what has been said, it must be here emphasized that the difficulty of detecting the presence of pathogenic bacteria in water is very great, and the length of time necessarily consumed in making the tests greatly lessens the value of the results when obtained. Added to this is the further limitation of the value that a negative result, *i. e.*, where no pathogenic bacteria found, cannot be taken as proof that the water-supply under examination may not be contaminated

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*Prescott. Report of American Public Health Association. Vol. XXIX., 356. Clark and Gage. *Ibid.* 386. Bissell. *Ibid.* 360.

†*Journ. Infect. Dis.*, Sup. No. 3. May, 1907. p. 30–32.

‡*American Journ. of Public Hygiene.* Vol. XVII., No. 4, Nov., 1907, p. 367.

§Consult Vaughan. *Journal American Medical Association.* April 9, 1904. For special methods of detecting the Bacillus coli communis see under this bacillus, page 310. For general subject of Water Supply and Public Health see Jordan. *Journ. Am. Med. Assn.*, V. XLVIII., May 18–June 8, 1907.

at times.* Flügge† has shown that the chemical examination also permits of no conclusion of itself as to the potability of water It would seem that those best suited by training and experience and who are capable of forming disinterested opinion attach but limited importance to the result of laboratory examinations of water unaccompanied by a sanitary inspection. Opinions based upon analyses of water shipped to a chemical or bacteriological laboratory should be taken with reserve. In fact, many of those who have made disinterested study of the subject are inclined to question the value of chemical and bacteriological water analysis *in toto*, and in view of the arbitrary and mechanical manner in which the results of these analyses are sometimes interpreted, this attitude is justified. It would seem, however, that after the establishment of normal standards for a given locality, such analyses are useful if they are checked by intelligent consideration of all the conditions entering into the case, but no hard and fast rules can be applied.‡

Ice.—The bacteriological examination of ice differs in no respect from that of water. Although development may be arrested, the vitality of bacteria is not necessarily impaired by freezing. Prudden found the bacillus of typhoid fever alive in ice after more than one hundred days. However, Sedgwick and Winslow have stated that when typhoid bacilli are frozen in water the majority of them are destroyed.§ Nevertheless, it is as necessary to have the source from which ice is taken as carefully scrutinized as that of the water-supply, especially in view of the universal habit of cooling water in the summer time by adding ice directly to the water. It is better

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*Gunther *loc. cit.* 283.

†Flügge. *Zeitschrift für Hygiene.* Bd. 22, 1896, pp. 445 et seq.

‡ Bolton. Sanitary Water Supplies for Dairy Farms. Public Health and Marine Hospital Service. Bulletin 41, February, 1908, p. 534.

§Clark. Bacterial Purification of mater by Freezing. Reports American Public Health Association. Vol. XXVII. See also Hutchings and Wheeler. *American Journal Medical Sciences.* Vol. CXXVI., p. 680

to cool water and articles of food by surrounding the vessels containing them with ice.

Bacteria of Milk and Other Foods.*—Of the different food substances, milk is probably the most important from a bacteriological point of view. In the first place, most other foods are cooked before eating. Furthermore, cow's milk constitutes the principal food of young infants, who are highly susceptible to certain bacteria and to substances in the milk itself, after it has undergone certain alterations due to bacteria. The milk of the healthy cow as it is secreted in the mammary gland is sterile; however, after milking the cow a little milk generally remains in the milk-ducts and in the lower part of the teat in which numerous bacteria will have developed before the next milking-time.* The first milk obtained at a milking should therefore be discarded, as it may contain an excessive number of bacteria.

In examining milk for bacteria the number may be estimated by precisely the same technic as is used for the estimation of the number of bacteria in water. The milk requires to be diluted usually more than water for the reason that there are a great many more bacteria ordinarily present in milk than in water, and consequently a very small amount of milk has to be used, so small that it cannot be accurately measured except by diluting. If much milk is introduced into the culture medium it makes the latter opaque.

A committee of the American Public Health Association† recommend, among other things, the following: Milk should be plated within four hours after it is collected, and in the meantime should be kept below 40° C., but not frozen. Dilutions recommended are 1–10, 1–100, 1–1000, 1–10,000, 1–100,000,

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*See Conn. Bacteria in Milk and its Products. 1903. Russell. Dairy Bacteriology. 1906. 7th Ed.

†Preliminary statement by the committee on standard methods of bacterial milk analysis. *American Journal of Public Hygiene.* Vol. XVII., No. 4, November, 1907.

1–1,000,000. The 1–10, dilution should be prepared by shaking the sample twenty-five times and taking 1 c.c. and putting it into 9 c.c. of sterile water. The 1–100, dilution is prepared in the same way, adding 1 c.c. of the milk to 99 c.c. of sterile water. The other dilutions are prepared from the 1–1000 dilution. The plate giving from 40 to 400 colonies should be selected for counting. All the colonies on the plate should be counted in preference to counting any part of a plate and calculating the total number. The culture medium should be agar, containing 1 per cent. agar, and it should be made from the watery extract of beef. The reaction should be + 1.5,* American Board of Health Scale. The majority of those consulted by the committee count the colonies after twenty-four hours, incubation at 37° C. in a moist incubator.

As a routine procedure, in cold weather, entirely satisfactory results may be obtained by taking 1 c.c. of the milk to be examined after it is thoroughly mixed, and putting it into 9 c.c. of sterile water, and taking 1 c.c. of this solution in 9 c.c. of sterile water. Plates made from this dilution with 1–10 c.c. and with 1 c.c. respectively have been found to give closely corresponding results, and unless the milk is badly contaminated it is always possible to count the colonies readily. In warm weather and in the case of cream, a third or even a fourth dilution should be made. Where the milk or cream are mixed with the medium in the tube, the resulting colonies are apt to be more uniformly distributed on the plate than where the milk is put into the Petri dish and the culture medium poured in afterward. The number of bacteria remaining in the test-tube in the former method of proceedure must be very few where the medium is properly fluid when it is poured. The objection to this method would appear to be purely theoretical, and counterbalanced by its advantages.

Contamination of the milk may occur from the outer surface of the udder of the cow, from the hands of the milker, from dirty pails, through agitation of the air of the stable, and in other ways. In view of these sources of contamination it is not to be wondered at that the number of bacteria found ordinarily in milk is usually very large. In ordinary milk as furnished by milkmen the number of bacteria to the cubic centimeter is usually many thousands, and may run up to many millions. Milk as obtained from the grocers, where it is apt not to be kept properly cooled, frequently contains hundreds of thousands or many millions of bacteria to the cubic centimeter. Many or most of these bacteria must be little if at all detrimental to health, otherwise milk would be entirely unsuitable as food in the raw state; whereas this is not the case. On the contrary, there are some bacteria which are useful in the manufacture of dairy products. The ripening of cream and of cheese is due to the growth of bacteria introduced in cultures or, what amounts to the same thing, it is due to the growth of bacteria introduced in some previously ripened cream or cheese. This imparts the agreeable flavor. Moulds are also used to give the peculiar flavor to some cheeses.*

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*See page 67.

But aside from these more or less innocuous forms and those which are useful, milk may at times contain pathogenic bacteria.

Tuberculosis is a disease to which cattle are exceedingly prone. There is good reason to believe that infants acquire tuberculosis through taking as food the milk of tuberculous cows, although the danger from this source has probably been overestimated. The milk of tuberculous cows may contain tubercle bacilli when there is no tuberculous disease of the udder.† The frequency of tuberculosis among milch cows sometimes becomes as high as 25 per cent., or even more. Butter derived from the milk of such cows may contain tubercle bacilli. The proper manner for the States to deal with this problem, for it is one that doubtless will fall to the individual States, has not yet been determined. The cost of killing such a large number of valuable cows would be very great. Furthermore, it is by no means certain that this procedure would eradicate the disease. The flesh of cattle also is capable of transmitting tuberculosis, but is a less serious source of danger when beef is thoroughly cooked.

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*Conn. Agricultural Bacteriology.

†Mohler. Infectiveness of milk of cows which have reacted to the tuberculin test. U. S. Department of Agriculture, Bureau of Animal Industry. Bulletin No. 44, 1903.

Epidemics of typhoid fever and cases of diphtheria have been traced to milk; and no doubt cholera asiatica is conveyed in this way in times of epidemic.

Human milk often contains streptococcus epidermidis albus, and not seldom the staphylococcus pyogenes aureus under normal conditions.

Scarlet fever is probably conveyed by milk, but as the organism causing this disease is not yet definitely known, it has not yet been detected in milk. But there is apparently good clinical evidence of this.

Streptococci have been found quite frequently in milk sold in the market.* Bacillus coli communis is very often present in milk, but it is probably without significance unless it is present in very large numbers when it is possible that it causes injurious fermentative changes. Fermentative changes are also caused by the presence of bacteria which are not in themselves the cause of disease, and these changes may render the milk unfit for consumption or even poisonous. These alterations may be evident to the senses, as the ordinary lactic acid fermentation (souring of milk), or they may not. The character of the alterations doubtless varies much with the temperature and with the character of the contaminating bacteria. Summer temperatures of course favor decomposition and fer-

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*Reed and Ward. The Significance of the Presence of Streptococci in Market Milk. *American Medicine.* February 14, 1903.

mentation. Specialists in children's diseases attribute to alterations in milk with the formation of poisonous substances a preëminent influence in the production of the intestinal disorders of infancy so common in the summer.

Poisoning with milk, ice-cream or cheese is not rare, as is well known. There are many records of whole companies of individuals having been taken violently ill after having eaten one of these foods from the same source of supply. The symptoms in such cases resemble those produced by irritant mineral poisons such as arsenic: nausea and vomiting, vertigo, dryness of the mouth, sense of burning and constriction in the throat, difficulty in swallowing, cramps and griping pain in the bowels, constipation or diarrhea, general prostration or even collapse. Vaughan isolated from poisonous cheese a ptomaine which he called tyrotoxicon. It appears, however, that other toxins may be present in cheese, and that tyrotoxicon is a somewhat rare poison. Vaughan holds that bacteria of the colon group play an important part in producing poisons in milk and cheese.

To prevent the alteration by bacteria of milk intended for the food of infants, the practice of sterilizing milk has been largely in vogue. Unfortunately, during sterilization the milk undergoes some kind of alteration which makes it disagree with certain infants. Furthermore, organisms possessing very resistant spores—the hay bacillus and the potato bacillus—are apt to be present in milk, and these are not killed by any process to which the milk should be subjected for infant feeding. Least of all does sterilization purify milk in which bacterial poison has already formed.

In regard to the destruction of the tubercle bacillus in milk Günther* has this to say: Morgenroth found that 10 minutes' heating at 70° C. does not kill the tubercle bacillus, and neither does 2 hours at 55° C. The same authority found that in order to destroy the tubercle bacillus in milk from a tuberculous cow

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Loc. cit.

containing the tubercle bacillus it is necessary to heat the milk for 3 hour at 55° C. or 30 minutes at 70° C., or from 3 to 5 minutes at 100° C. Bang found that 2 minutes, heating at 60° C. caused a marked diminution in the virulence of the tubercle bacilli in milk. Morgenroth found that a very short heating at 100° C. sufficed to diminish the virulence materially.

Rosenau* found that in milk the tubercle bacillus loses its virulence and infective power, *i. e.*, is killed, by exposure to 60° C. for twenty minutes.

The investigations of Park and Holt† show that in New York City the number of bacteria in milk is much smaller in winter than in summer, and has little effect on the health of infants during cold weather; but that in warm weather with milk of average quality the infants who received sterilized milk throve on the average much better than those who received raw milk.

The process called pasteurization is designed, not to sterilize the milk completely, but to destroy the vegetative forms of bacteria, and to destroy the ordinary pathogenic bacteria with which the milk might possibly be contaminated.‡ The milk is subjected to a temperature of only about 70° to 75° C. This temperature is less likely to produce alteration in the milk than sterilization by steam at 100° C. According to Freeman, milk which had been pasteurized at 75° C. and distributed among the poor people of New York City, whose homes were not supplied with ice, usually kept very well even in the summer time.

The number of bacteria in milk may be reduced considerably by the use of the centrifuge (separator).

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* Bull. No. 42, Hyg. Lab., U. S. Pub. Health and Mar. Hosp. Serv., Wash. pp. 1–85. Jan., 1908.

†*Archives of Pediatrics.* December, 1903.

‡Theobald Smith. The Thermal Death-point of Tubercle Bacilli in Milk, etc. *Journal of Experimental Medicine.* Vol. IV., p. 217.

St. John and Pennington* conclude from their study of the effects of pasteurization of milk, that there is a restraining power in raw milk lasting at least to the curdling point which is destroyed or reduced in milk heated to 79° C.

Rosenau,† from a consideration of the various statements made in favor of and those which are against pasteurization, comes to the conclusion that heating of milk has certain disadvantages which should be given due consideration, but since it is the means of saving many lives of children, particularly during the summer months, pasteurization is to be recommended. The ideal condition would be milk obtained so clean and kept so cold that there would be no necessity for pasteurization, but such conditions are not found in practice. Rosenau does not regard the statement that pastuerization "devitalizes" the milk as having any force or indeed any exact meaning. He does not regard the idea that pasteurized milk is a cause of scurvy as well founded. Under conditions as they exist, the consensus of opinion among those best qualified to pronounce upon the subject seems to favor the pasteurization of milk, at least for infant feeding. Theobald Smith‡ points out that the objection raised by some to pasteurization that it conceals dirt is erroneous for the reason that the bacteria coming from dirt are spore-bearers, and are not killed by pasteurization. Rosenau further states that comparative observations have shown that children thrive quite as well on pasteurized as on raw milk, that pasteurized milk is in fact more easily digested than raw milk. But on the other hand, there are many who object to pasteurization of milk, and the question can hardly yet be

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*St. John and Pennington. *Journal of Infectious Diseases.* IV., No. 4, 1907, p. 655.

†Rosenau. Milk and its Relation to the Public Health. Pasteurization Bulletin 41. Hyg. Lab., U. S. Pub. Health and Mar. Hosp. Serv. Wash., D. C., pp. 589—624.

‡Theobald Smith. *Am. Journ. Pub. Health* and Journ. Mass. Assn. Bds. Health. Vol. 17, 1907, p. 200. Quoted by Rosenau., *loc cit.*

regarded as settled. Nevertheless, the advantages seem certainly to greatly outweigh the disadvantages.

In regard to the pasteurization of milk, Rogers* has this to say:

"Examination of milk by many bacteriologists shows that the milk used in American cities is usually badly contaminated by bacteria.

"Increased public interest in the milk-supply has resulted in more rigid municipal regulations and inspection, but progress is necessarily slow and pasteurization is frequently resorted to in order to increase the length of time that the milk will remain sweet and to reduce the danger from spread of infectious diseases.

"The objection is frequently made that pasteurization, by destroying the lactic-acid bacteria, allows the development of other less desirable bacteria which, without affecting the taste of the milk, make it actually dangerous, especially as a food for young children.

"It is well established, however, that under certain circumstances the intestinal troubles of children may be reduced by pasteurization of milk.

"Milk was pasteurized under laboratory conditions in a continuous machine at 85° C. (185° F.), the bacteria being reduced from over 10,000,000 per cubic centimeter to less than 500 per cubic centimeter.

"*Milk held at* 20° *C.* (68° *F.*).—In the unheated milk the lactic bacteria increased rapidly and the milk became acid in about 12 hours. The peptonizing bacteria increased in 6 hours to about 5,000,000 per cubic centimeter and then decreased slowly.

"In the heated milk the peptonizing bacteria increased rapidly after 12 hours, and the milk was usually curdled in 48 hours, with a disagreeable taste and odor. Occasionally lactic bacteria survived pasteurization and multiplied rapidly after 24 hours, completely inhibiting the peptonizing bacteria.

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*Bureau of Animal Industry. Bul. No. 73, 1905

"*Milk held at* 10° *C.* (50° *F.*).—In unheated milk the growth of bacteria and the consequent curdling of the milk was much retarded. The average milk did not contain sufficient acid to affect the taste until it was over 48 hours old. The proportion of peptonizing to lactic bacteria was greater than at the higher temperature and the taste of the milk occasionally showed the influence of the former.

"In the pasteurized milk the bacteria increased very slowly, and in nearly every case the milk was unchanged in taste and appearance 96 hours after pasteurization. In only two of fourteen cases was there a marked increase of peptonizing bacteria. The predominating bacteria were species having little or no effect on milk.

"The lactic bacteria inhibited the development of the peptonizing bacteria only when they had developed sufficient acid to render the milk unfit for use.

"It seems probable that the acid had a distinct inhibitory action on the proteolytic enzymes of the peptonizing bacteria.

"If milk could be pasteurized commerically in such a way that the bacteria would be reduced to a few hundred per cubic centimeter and held at a low temperature until used, it would be perfectly safe for 48 hours or even 72 hours. Under these circumstances it would probably be in better condition after this long period than ordinary city milk at the time it is delivered. How closely these conditions could be approximated commercially is another question."

Since the publication of the above bulletin, it has been demonstrated in some dairy plants that such conditions can be brought about upon a commercially profitable scale, and it would seem justifiable on the part of health authorities to demand that milk dealers comply, or that the municipal authorities should establish pasteurizing plants.

The great interest which is taken by the public and by physicians in the subject of pure milk has led some dairyman to take steps to prevent contamination of the milk by cleanliness in the barnyard and stable, and in careful cleansing of the udders of the cows, the hands of the milkers and the milk-cans. The dairy-rooms are also kept scrupulously clean in the better class of dairies, and the floors are kept wet to avoid dust* The use of ice in cooling the milk as soon as it is drawn, and in transportation is also used, and this serves to prevent the multiplication of bacteria. In many dairies the cattle from which the milk is obtained are regularly inspected at intervals by veterinary surgeons as well as subjected periodically to the tuberculin test. The surroundings and drainage of the stables are investigated by physciians and sanitary engineers. The milk is also regularly analyzed by a chemist and bacteriologist. It has been found possible by such precautions to reduce the number of bacteria in milk to a few thousands per cubic centimeter or even much less.

Other articles of food which are eaten after little or no cooking such as salads, green vegetables, fruits, and the like, may become, under exceptional circumstances, agents for conveying infectious diseases. Conn showed that there was good reason for attributing an epidemic of typhoid fever among students at Middletown, Connecticut, to raw oysters. After having been collected from the oyster-beds, these oysters were placed in a small stream to fatten, where they were exposed to contamination from a sewer. Into this sewer the discharges of a case of typhoid fever were found to have been running at the time when the oysters were fattening. An epidemic at Atlantic City, New Jersey, in 1902, was traced to nearly similar causes and conditions.†

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*W. H. Park. *Journal of Hygiene.* Vol. I., 1901.
†*Philadelphia Medical Journal.* November 1, 1902.

The ordinary processes for curing and salting meat cannot be relied upon to destroy pathogenic bacteria.

Cases of botulism, or poisoning by eating oysters, fish, meat in the form of sausage or canned meat, and other articles of food, are not rare. They are due to products of bacterial decomposition, as in the case of those poisoned by milk and cheese already mentioned. Such affections are quite commonly called ptomaine poisoning. A number of bacteria exist which are capable of affecting injurious changes in meat and other foods either before or after ingestion. Among these are an anaerobic bacillus described by Van Ermengem (B. botulinus), various members of the groups represented by B. proteus and B. coli communis (including paracolon bacilli), and the bacillus enteritidis of Gaertner. In the case of B. enteritidis a genuine infection of the patient and gastroenteritis may occur.*

*See Vaughan and Novy. The Cellular Toxins. 1902. Ohlmacher. Food Intoxication from Oatmeal. *Journal of Medical Research.* Vol. VII., p. 420. Galeotti and Zardo. *Centralblatt für Bakteriologie.* Vol. XXXI. 1902. Orig. p. 593.

CHAPTER IV.

THE BACTERIA OF THE NORMAL HUMAN BODY.

ALTHOUGH there is considerable discrepancy in the results of various investigations in regard to the matter, it would appear that the solid tissues of the animal body, the blood and lymph, and the cavities that have no connection with the outer world, are often free from bacteria.* So also the maxillary, ethmoidal and frontal sinuses, middle ear,† urinary bladder, uterus and Fallopian tubes, and to a less extent the lungs‡ and gall-bladder,§ although having external connections, are usually sterile when in a healthy condition. When bacteria do enter the tissues from any of the surfaces their progress is checked by means of the activities of the cells or fluids of the body, and if they succeed in penetrating to any considerable distance their advance is usually arrested by the nearest group of lymph-nodes, which appear to be important safeguards for preventing the dissemination of bacteria throughout the body. As a rule, the sections of the mucous membranes are inimical to bacteria.

The skin,|| as might be expected, is liable to have upon it

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*Ford found small numbers of bacteria in the normal organs of rabbits, cats and dogs in the majority of those examined. The species of bacteria obtained were mostly common saprophytes, and to some extent constant in the same kind of animal. *Journal of Hygiene.* Vol. I. 1901.

†Calamida and Bertarelli. *Centralblatt für Bakteriologie.* Vol. XXXII. 1902. Orig. p. 428. Törne. *Ibidem.* XXXIII. 1903. p. 250. Hasslauer. *Ibidem.* Referate. XXXII, p. 174. An examination of these articles will show that investigators disagree somewhat, with regard to the sterility of these cavities.

‡See Wadsworth. *American Journal Medical Sciences.* May, 1904.

§See Bacteriology of the Gall-bladder and its Ducts. *American Journal Medical Sciences.* Vol. CXXIII., p. 372.

||Sabouraud. La Peau Humaine, etc. *Bulletin de l' Institut Pasteur.* II 1904. Pages 233, 282.

numerous bacteria, especially micrococci, and moulds. The staphylococcus pyogenes aureus, the streptococcus pyogenes, the bacillus pyocyaneus and the bacillus coli communis sometimes occur on the skin. According to Welch, it always contains the staphylococcus epidermidis albus, which may be a form of the staphylococcus pyogenes albus. This organism is of some importance to surgeons on account of its relation to the cleansing of the skin before operations. It seems impossible, by any amount of cleaning, to dislodge all of the germs in the skin especially those under the nails.

The bacteria of the exposed mucous membranes like the conjunctiva* and the nasal cavity† and the mouth cavity naturally fluctuate both in quantity and quality; they consist, in fact, of those which happen to fall upon the surface or are drawn in from the external air.

In the mouth, however, there is a certain group of organisms more or less characteristic of it, many of which have not been successfully cultivated. These have been thoroughly studied by Miller, to whose works students are referred.‡

Several species of spirilla have been discovered in the mouth and are found along the margins of the gums. The leptothrix buccalis, and related organisms which have a long, ribbon-like form, also occur in the mouth. The micrococcus lanceolatus (or pneumococcus) is present in many human mouths. In 15 to 20 per cent. of human mouths this organism is sufficiently virulent to produce fatal septicemia when inoculated into susceptible animals. Pyogenic bacteria, especially streptococci occur frequently, although not regularly, in the mouth. Streptococci very commonly occur on the tonsils. Putrefactive

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*Randolph, Pusey, Gifford. *Journal American Medical Association.* Oct. 3, 1903.

†Hasslauer. Die Bakterienflora der gesunden und kranken Nasenschleimhaut. *Centralblatt für Bakteriologie.* Vol. XXXIII. 1902. Orig. p. 47.

‡Miller. Microörganisms of the Mouth. For a review on the bacteria of the mouth, see Madzar. *Centralblatt für Bakteriologie.* Vol. XXXI. Ref. p. 489. Vol. XXXII, p. 609.

bacteria acting on particles of food about the teeth produce the bad odor from the mouths of persons of careless habits. According to Miller, bacteria play an important part in the production of dental caries. Certain of the bacteria of the mouth produce fermentation in the vicinity of the teeth with the formation of acids, which dissolve the calcium salts of the teeth. The softening and destruction of the decalcified matrix is then accomplished by other forms.

The expired air coming from the mouth and nose, contrary to the popular notion, is free from bacteria, excepting those which become forcibly detached, as by efforts of sneezing and coughing.

McKee* found that in the great majority of normal conjunctivas the ordinary pyogenic bacteria and the bacillus xerosis are present. He quotes Eyre† as saying that the conjunctival sac frequently contains bacteria which may or may not be pathogenic; but, on the other hand that it may be sterile, due to the mechanical flushing of the mucous surface by the lachrymal secretion, aided perhaps by the bactericidal property of the latter.

Among the other exposed mucous surfaces, the urinary meatus and the vagina may be included. The urinary meatus and at least part of the urethra will be found to contain bacteria, which, in health, should be non-pathogenic, although interest attaches to the fact that diplococci have been described which behaved with stains in the same manner as the gonococcus (pseudogonococci).

There has been much dispute as to whether or not the pyogenic bacteria occur in the vagina normally. But there appears abundant evidence going to show that while the vagina may be free from pathogenic bacteria it often if not usually harbors pyogenic bacteria. While it is true that the normal

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*Reprint from *Montreal Med. Journ.* Jan., 1906.

†*Lancet.* Dec. 21. 1895. *Journ. Pathol. and Bacter.* July, 1896.

secretion of the vagina has a bactericidal influence which may be attributed in part to its acidity, this does not seem to be effective at all times. The upper part of the normal cervix uteri is sterile, while bacteria are present in the lower part.

McDonald* quotes from Bumm and Sigwart to show that streptococcus is present in the secretions from the uterus in 38 per cent. of cases in women during the latter stages of pregnancy, and that they further more state the belief that this organism is present in 75 per cent. in such cases. That of the cases showing the presence of streptococci, 24 per cent. had fever. She agrees with the authors cited that the presence of streptococcus is not sufficient grounds for a diagnosis of puerperal infection. The futility and harmfulness of curettment in cases of general infection is evident.

According to Döderlein, the properties of the vaginal secretion are due to bacilli which very commonly occur in it. The secretion is most abundant and important during pregnancy.†

The smegma of the external genitals contains numerous bacteria, among which are frequently found bacilli which retain their color after treatment with acids in the Gabbett method for staining tubercle bacilli. It is uncertain whether these bacilli form a special group of organisms by themselves, having as one of their properties the power of retaining the stain after acids, or whether they are bacilli of no particular sort, which resist acids after staining owing to the oily material with which they have been impregnated in this peculiar secretion. These organisms must be taken into account in examining urine or other secretions for tubercle bacilli, for particles of smegma might be accidentally present. Usually the employment of alcohol after the acid will remove the color from the smegma bacilli (Hueppe). Sometimes smegma bacilli are as resistant as tubercle bacilli to decolorizing agents (Welch) (see page 34). Similar acid-proof bacilli occur about the genitals of the domestic animals.‡

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*Reprint from *American Medicine*. Vol. XI., No. 7. Feb. 17, 1906, pp. 231–238.

†J. W. Williams. Obstetrics. A Text-book, etc. 1908. Wadsworth. *American Journal of Obstetrics*. Vol. XLIII. 1901.

‡Cowie. *Journal Experimental Medicine*. Vol. V., p. 205.

Bacteria are always present to a greater or less extent in the stomach and intestines except for a few hours after birth. The alimentary tract of new-born infants and the meconium are sterile. In from four to eighteen hours organisms begin to appear. They may enter either from the mouth or the anus. There seems to be no constancy in the nature of the forms which are found at first, but their character depends upon the surroundings.

The species of bacteria found in the stomach are less constant than those of the intestines; and under normal circumstances they seem to be those introduced from the mouth. Different investigators, at all events, have met with quite different species in the stomach. It appears that the hydrochloric acid (about 2 parts per thousand) present in the gastric juice at the height of digestion possesses decided germicidal properties. This germicidal power exercises a restraining influence upon fermentation due to bacteria, and probably serves as a safeguard against the introduction of pathogenic germs into the intestines. That is particularly important in the case of the spirillum of cholera, which is excessively sensitive to the action of acids. Nevertheless, many bacteria are able to reach the intestines uninjured, as the acidity of the gastric juice does not reach its height until some hours after eating. Such bacteria will be those which are most resistant and those which form spores. In the intervals when hydrochloric acid is absent from the stomach, lactic acid appears. It is formed from carbohydrates by a large number of species of bacteria. In conditions of fermentation, sacrina ventriculi and yeasts may be present in large numbers; in the healthy stomach they occur in much smaller numbers.

The intestine of the infant in whom feeding has become well established was found by Escherich to contain two principal species of bacteria—in the lower part of the intestine the bacillus coli communis, in the upper part the bacilus lactis aërogenes. More recently it has been shown that the stools of milk-fed infants, and to a less extent of adults, contain large numbers of anaerobic bacilli, which stain by Gram's method (bacillus bifidus—Tissier, bacillus acidophilus—Moro). These bacteria have not been fully studied*.

The number of bacteria in a milligram of human fecal matter has been estimated at from seventy thousand to thirty-three million.† It is estimated that about one-third of the fecal matter of adults if dried would be found to consist of bacteria.‡ The small intestine of adults has been found by different observers to contain very different species.§ The majority of these appear to have been introduced from the mouth in food or water. The bacillus coli communis, however, occurs invariably in health not only in the intestine of man, but also in that of many animals, especially in the lower part.|| The pyogenic micrococci very often occur in the intestine.

In the case of ruminant animals like the cow and sheep, the decomposition of cellulose, which forms so large a part of their food, appears to be affected by bacteria. Bacteria having this power are constantly found in the stomachs of ruminants. The best known species is that called bacillus amylobacter. It is questionable whether the products of the decomposition of cellulose have any nutritive value.

Pasteur some years ago expressed the opinion that if animals could be placed in such surroundings that bacteria could be excluded from the alimentary canal and the food, life would be impossible. This view has excited much controversy, and was apparently disproved by the experiments of Nuttall and Thierfelder.

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*Metchnikoff. Les Microbes Intestinaux. *Bulletin de l'Institut Pasteur.* May 15 and 30, 1903.

†Our method for estimating the number of bacteria in feces see Steele. *Journ. Am. Med. Assn.*, XLIX., Aug. 24, 1907, p. 647.

‡*Strassburger Centrallbel. f. Bakt.*, etc. Ref. Bd. 32. p. 561.

§Ford. Classification of Intestinal Bacteria, etc. Studies from the Royal Victoria, Montreal. March, 1903.

||Moore and Wright. Bacillus coli communis from Certain Species of Domesticated Animals. *American Medicine.* March, 1902.

These investigators succeeded in removing guinea-pigs from the mother by Cesarean section, and in keeping them alive in sterile surroundings, upon sterile food, so that the contents of the alimentary canal remained sterile. Schottelius, who worked with chickens, obtained contrary results, however; so that this interesting question is still undecided

CHAPTER V.

BACTERIA IN DISEASE.

To the physician and the student of medicine the study of bacteriology is interesting chiefly on account of the great importance attributed to bacteria in producing disease. The presence in an organism of one or a number of organisms of another species, which flourish as parasites upon the first, is a phenomenon of very wide occurrence in nature. It is, in fact, nearly universal. It may be observed among plants as well as animals, for example in the familiar galls seen on some of the higher plants, and mostly caused by the larvæ of insects harbored by the plant. We also find animals, such as tapeworms and the trichina spiralis, living as parasites upon other animals. The conditions favorable to the growth of certain bacteria make them peculiarly suited to leading a parastic existence. The fact that they possess no chlorophyll, and that they are therefore unable to form carbon compounds from the carbon dioxide of the atmosphere, renders it necessary for them to secure such compounds from pre-existing organic matter. Most of them, furthermore, flourish better when they are able to obtain nitrogenous food from organic matter rather than from inorganic salts containing nitrogen. Most bacteria, those known as saprophytes, find the necessary nutriment in the dead bodies of other animals and plants; but some of them, those known as parasites, flourish upon the living bodies of other plants and animals and produce disease.

The phenomena of disease, as has been well established, are due in a number of cases to the numerous waste products of the activities of bacteria, which act as poisons to the host.

The diseases of plants known to be caused by bacteria are not very numerous. Among them may be mentioned pear-blight, due to micrococcus amylovorus.* Among lower animals bacteria very frequently produce diseases—for example, chicken-cholera, symptomatic anthrax in cattle, erysipelas of swine, tuberculosis, anthrax and glanders in various animals, "red leg" in frogs.†

These are some of the diseases in which bacteria have been shown definitely to be the cause. It is not enough in any case merely to find bacteria to establish the connection between them and the disease. Koch's postulates, as they are called, given below, must be complied with in order to prove that any microorganism is the cause of a particular disease:

First. That the organism should always be found microscopically in the bodies of animals having the disease; that it should be found in that disease and no other; that it should occur in such numbers and be distributed in such a manner as to explain the lesions of the disease.

Second. That the organism should be obtained from the diseased animal and propagated in pure culture outside of the body.

Third. That the inoculation of these germs in pure cultures, which had been freed by successive transplantations from the smallest particle of matter taken from the original animal, should produce the same disease in a susceptible animal.

Fourth. That the organism should be found in the lesions thus produced in the animal.

A moment's consideration will show that it is impossible to comply with all these postulates in the investigation of all infectious diseases; for in some cases the organisms causing the diseases have not yet been observed, and yet there is abundant proof that they exist in certain tissues of animals suffering from

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*See E. Smith. *Centralblatt für Bakteriologie*, etc. Zweite Abtheilung. Bd. V., p. 271; Bd. VII., p. 88.

†Norris and Emerson. *Journal of Experimental Medicine*. Vol. VII., p 30.

these diseases. Thus, it is well known that the hydrophobia virus is to be found constantly in the brain and cord of animals suffering from rabies, and yet it has been impossible to isolate and cultivate the organism. Again, the leprosy bacillus is found always present in the lesions of the disease, and yet it has not yet been cultivated and reinoculated. The spirocheta pallida of syphilis has been successfully inoculated from syphilitic lesions into monkeys, but it has not yet been cultivated outside the animal body.

Still, although the etiology of some diseases seems firmly established even where all of these postulates have not been fulfilled, it is nevertheless the aim in all cases to comply with them as fully as the nature of the case, and the limits of present knowledge and technique will permit.

If the fact is once established in any given case that a disease may be communicated from a sick individual to a healthy one it is classed as an infectious disease, and infectious diseases are all caused by some living parasite. Consequently:

An *infectious disease* is a disease which is caused by a microorganism growing in the body of the animal having the disease. Such microörganisms are usually bacteria, but not always; for example, malaria is produced by a minute animal organism.

A *contagious disease* is one which is acquired from direct or indirect contact with an individual having the disease. Most contagious diseases are infectious, but infectious diseases are not necessarily contagious. The words are often used very loosely, and it is no longer possible or very desirable to draw the line sharply between them. *Fomites* are the materials on which the infectious material is conveyed.

A *miasmatic disease* is a variety of infection in which the microörganisms are not received from another case of the disease, but are supposed to have been derived from the external world, particularly through foul air. This word is less used than formerly.

The following is a list of the most important diseases of man caused by bacteria. The proof as required by the rules of Koch is not complete for all of them:

Tuberculosis,
Leprosy,
Glanders,
Anthrax,
Tetanus,
Malignant edema,
Bubonic plague,
Malta fever,
Erysipelas,
Suppuration and certain inflammatory conditions allied to it,
Epidemic cerebrospinal meningitis,
Gonorrhea,
Chancroid or soft chancre,
Lobar pneumonia,
Influenza,
Diphtheria,
Typhoid fever,
Dysentery (not amebic dysentery),
Asiatic cholera,
Relapsing fever,
Actinomycosis.
Syphilis (probably).

Malaria and amebic dysentery are caused by microscopic unicellular animal organisms (protozoa). It has been claimed that small-pox is caused by protozoa; this view has acquired added interest from the recent researches of Councilman. Recent work indicates that the "sleeping sickness" (of Africa) and some other diseases of tropical climates are caused by protozoa (see appendix).

Thrush and certain parasitic skin diseases are caused by fungi of more highly organized structure than bacteria.

In each of the following diseases there is good reason to think that the cause is some kind of microörganism, but it has not yet been discovered:

Chicken-pox, measles, scarlet fever, German measles, mumps, whooping-cough,* yellow fever, typhus fever, rabies, dengue.

Rheumatic fever and beri-beri would be placed in this list by many writers.

Bordet and Genou have isolated an organism in whooping-cough from sputum by the use of a mixture of rabbit's blood with agar and a small amount of glycerin extract of potato. They state that another organism resembling the influenza bacillus often presents a serious obstacle to the isolation of the real whooping-cough bacillus. *Ann. de l'Inst. Past.* XXI., No. 9, Sept. 25, 1907. pp. 727–732.

The causes of these diseases have been very carefully sought for by ordinary bacteriological methods with indecisive results. Some of them no doubt are due to bacteria. In recent years numerous observers have described a diplococcus or short streptococcus as the cause of rheumatic fever or acute rheumatism. In the case of yellow fever Sanarelli described an organism (bacillus icteroides) as its cause, but his view is not upheld by most of those who have worked on yellow fever.* The bacillus described by a number of observers as having been found in cases of whooping-cough may also be the cause of that disease.† Bacilli have also been described in cases of measles on several occasions. The organism obtained by Lustgarten and that obtained by Joseph and Piorkowsky in the lesions of syphilis are no longer regarded as the cause of the disease, but the spirocheta pallida of Schaudinn and Hoffmann is probably the cause.

It is likely that for some of the diseases mentioned other procedures than the usual methods of research will have to be devised in order that the cause may be discovered. The protozoa may play a part in the etiology of some of them. Roux has produced evidence to show that contagious pleuropneumonia of cattle is due to a microbe so minute that it is barely visible with the highest powers of the microscope, so that its outlines and its morphology cannot be studied. The virus of this disease remains virulent after being passed through a Pasteur filter, showing that it is small enough to go through its pores. Similar experiments have succeeded with a number of other affections of animals; for example, foot-and-mouth disease and hog cholera or, at any rate, an infectious epidemic disease presenting all the features of hog cholera. The virus may pass thorugh a Pasteur or Berkefeld filter of a certain coarseness, but

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*Sanarelli. *La Semaine Médicale.* April, 4, 1900. Reed and Carroll. *Journal Experimental Medicine.* Vol. V.

†See Czaplewski. *Centralblatt für Bakteriologie.* Bd. XXIV. 1898. p. 865.

may be restrained by one sufficiently fine. The virus of rabies is also probably filterable. Reed and Carroll found that the infective agent of yellow fever is in the blood, and that the serum could produce yellow fever in a non-immune person after filtration through a Berkefeld filter.* These facts suggest the possibility that failure to find the causes of some other diseases may lie in the fact that their organisms are so small as to be nearly or entirely invisible to the microscope.

Ashburn and Craig,† in a preliminary report on their researches into the etiology of dengue, state that so far as they have gone they have found that the causative agent, whatever its character, resides in the blood of persons suffering from the disease, since intravenous injections of human beings with blood from dengue fever patients produce the disease. The organism is probably ultramicroscopic, since it passes through a Pasteur filter. The incubation period in the persons injected is four days and it is the same whether filtered or unfiltered blood is employed. The disease, they state, is not contagious, but is conveyed by at least one species of mosquito, *Culex fatigans*.

They find on further study‡ that no organism, either bacterium or protozoön, can be demonstrated in the blood.

Modes of Introduction.—There are various avenues by which bacteria may enter the body to produce disease. Infection of the embryo through the ovum or semen seems to be of rare occurrence. Syphilis is transmitted in this manner. The embryo may be infected through the placenta, although not commonly. The bacilli of typhoid fever and the pus-forming bacteria have been known to be conveyed through it.

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*See Reed and Carroll. *American Medicine.* February 22, 1902. For an admirable review of this subject see Roux. Sur les Microbes dits Invisibles. *Bulletin de l'Institut Pasteur.* Vol. I., Nos. 1 and 2. Also Dorset. Invisible Microörganisms. United States Department of Agriculture, Bureau of Animal Industry, Circular No. 57. 1904.

†*Journn. Am. Med. Assn.* V., XLVIII. No. 8., Feb. 23, 1907. p. 693.

‡*Journ. Infect. Dis.* No. 3, Vol. 4. June 15, 1907. pp. 440–475.

Tuberculosis may also be transmitted through the placenta; how frequently is still uncertain. Occasionally the exanthematous fevers are transmitted from the mother to the fetus.

The surfaces covered with thick, stratified epithelium are not likely to be penetrated by bacteria except through some wound or other lesion. This, for instance, is true of the skin, the mouth, the vagina and bladder. The infection of bubonic plague appears to be introduced most often by means of wounds in the skin. Bacteria more easily penetrate surfaces having a thin, columnar epithelium such as occurs in the intestines, the middle ear, bronchi and bronchial tubes, uterus and Fallopian tubes.

The thin, flat epithelial cells of the air-vesicles of the lungs, as would be expected, seem to be passed with comparative ease. On epithelial surfaces covered with cilia, as in the bronchi and bronchial tubes, the Eustachian tubes, the uterus and Fallopian tubes, the current toward the exterior created by the cilia acts beneficially in removing bacteria.

The tonsils and lymph-follicles of the intestines, especially the lymphoid tissue of the ileum and the vermiform appendix, are points where bacterial invasion frequently begins. The lymphoid tissue of the appendix may have some influence in predisposing to infection at that point and to appendicitis. On the other hand, it is certain that the progress of many infections is checked by the lymph-nodes. That is repeatedly seen in the ordinary post-mortem wound where the spread of the inflammation along the arm is checked suddenly at the elbow or axilla. The participation of the lymphoid structures in most infections is well known. How far this is a conservative process it is impossible to say.

In most cases of infectious disease a point of entrance for the bacteria may be discovered. As a rule, the invading microbes produce a lesion at the point where they are introduced, as in the familiar cases of boils and carbuncles when pyogenic bacteria enter the skin, or of the tubercles found in the lungs when the bacilli lodge in the respiratory tract. However, there are cases of septicemia and pyemia in which the most careful search fails to reveal the place at which the bacteria entered. The bacilli of plague usually produce no reaction at the point of entrance.

Tubercle bacilli may pass through thin epithelial surfaces and lodge in the deeper structures underneath, where they produce definite lesions and leave no trace to mark the point of entrance. For example, they may pass by the lungs and enter the bronchial glands, and form tubercles in that situation.

Ravenel has shown that they pass through the walls of the intestines without causing any lesion there, and produce tuberculosis in the lungs and elsewhere.

Experiments on animals have shown that bacteria may be very rapidly disseminated after their introduction. The inoculation of mice, for instance, with anthrax bacilli has been known to prove fatal, although the wound was washed immediately with the strongest antiseptic solutions or the part amputated within a few minutes.

The agencies concerned in the transfer of infection have been referred to in Part II., Chapter III. In all cases, however, there is direct or indirect connection with another case of the same disease. W. H. Park was able to cultivate diphtheria bacilli from bedclothing soiled by the expectoration of diphtheria cases. Baldwin has shown that tubercle bacilli may be found on the hands of patients having pulmonary tuberculosis, especially those who expectorate on handkerchiefs. Winslow found bacillus coli communis on the hands of 5 per cent. to 19 per cent. of those examined; his observations suggest the possibility that typhoid bacilli can be carried in a similar manner.

Direct Contact.—Many diseases are conveyed from a sick individual to a healthy person by direct contact as in gonorrhea and syphilis. This mode of transfer is probably quite frequent in many diseases.

Healthy Persons as Carriers of Infection.—There are numbers of examples of infection having been spread by healthy persons who, though not suffering themselves, nevertheless harbor the infectious agent. This matter was prominently brought to light in the cholera epidemic in Hamburg, Germany, in 1892, where a number of persons who passed quarantine, and traveled to other parts of Germany were found to have the cholera spirillum in their feces. In some cases local outbreaks were traced to these persons. Other infectious diseases have been traced to the same source. Soper* has published the history of a case in which a healthy individual carried and disseminated the typhoid bacillus for years. Other cases of the same kind have been reported. Dehler† reports the case of a man who was a constant typhoid bacillus carrier on whom he operated for the sole purpose of cleaning out the gall-bladder to rid it of the typhoid bacilli. He afterward operated an another similar case for the same purpose, with the result that the bacilli disappeared from the stools. In regard to diphtheria there is abundant evidence that healthy persons may harbor the organism.

Air.—Excepting under certain special conditions, the air does not contain the germs of disease. Dried pulverized sputum containing tubercle bacilli may float in the atmosphere as dust. Flügge found that powerful expiratory efforts like coughing and sneezing may carry tubercle bacilli with small particles of secretion into the air, and that they may remain in suspension some time. The pus-producing bacteria may be present in dust. Infectious elements which happen to be present in the air are usually attached to particles of dust.

**Jouru. Am. Med. Assn.* June 13, 1907. p. 2019.

†*Munch Med. Wochenschr* Oct. 22, 1907. Abst. *Journ. Am. Med Assn.* Nov. 30, 1907. Also Eben. July 6, 1907, p. 94.

Wool-sorter's disease is a name sometimes applied to anthrax in man when acquired by inhaling the dust from wool which contains the anthrax bacilli or spores.

The atmosphere in the vicinity of cases of the exanthematous fevers must necessarily at times contain the germs of these diseases.

Water is commonly regarded as the usual medium for the transmission of the infection in typhoid fever, and Asiatic cholera, and probably all forms of dysentery besides various nondescript disturbances of the alimentary tract.

Milk from tuberculous cows may carry the bacilli of tuberculosis as already stated; this is, of course, of the utmost importance in the case of young infants. Typhoid fever and cholera, and probably dysentery, scarlet fever and diphtheria would all appear to be diseases which might be conveyed through the medium of milk and in some cases of these diseases this mode of infection has been quite clearly demonstrated. Not only milk but all other forms of uncooked food may serve as carriers of infection to the intestines.

The **Soil** is of importance as a mode of conveyance of infection because of the frequent presence in it of the bacteria of tetanus and of malignant edema. Bacillus aërogenes capsulatus may occur in the soil, and may infect dirty wounds. The spores of anthrax bacilli are present in the soil of certain localities, and may produce anthrax in cattle.

Flies.*—Under suitable conditions, flies play an important part in transporting the bacteria of cholera and typhoid fever from the excreta of these diseases to food substances, which they may contaminate. Flies which have access to tuberculous sputum may deposit tubercle bacilli on food.† Buchanan‡ has shown that the common house-fly and the blue-bottle fly are capable of carrying bacteria on their feet. The organisms experimented with were typhoid, swine fever, staphylococcal abscess, pulmonary tuberculosis and anthrax. L. O. Howard* and many others before and since have pointed out the same source of danger of spreading of infection. To what extent diseases are disseminated by fleas, bedbugs and similar creatures is still uncertain.

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*Nuttall. Rôle of Insects, etc., in Disease. John Hopkins Hospital Reports. Vol. VIII. 1900.

†Lord. *Boston Medical and Surgical Journal.* Dec. 15, 1904.

‡Circular 71, U. S. Department of Agriculture, Bureau of Entomology.

In this connection it is proper to refer to certain diseases due to animal microörganisms. Malaria is conveyed from man to man by mosquitoes of the genus Anopheles,† and is probably transmitted exclusively in this manner. The parasite of malaria undergoes part of its cycle of development in man, and another part in the mosquito. Similarly, in Texas fever, a disease of cattle, it has been shown by Theobald Smith that the parasite (a protozoön, Pirolasma) passes from cow to cow through the cattle-tick (Bophilus annulatus or bovis).‡ In surra, a disease chiefly affecting horses, and in the tsetse-fly disease of animals the parasite (a protozoön, Trypanosoma) is transmitted by the bites of flies.§ It has been shown that the infectious agent of yellow fever may be introduced into man by mosquitoes of the genus Stegomyia.|| Under the administration of the United States army yellow fever was suppressed in Havana chiefly by measures intended to prevent the disease from being carried by mosquitoes. Equally good results have since been attained in controlling an epidemic of yellow fever at Laredo, Texas, in 1903,¶ and a great reduction in the mortality at Rio Janeiro, Brazil, has been effected.

Auto-infection.—It is possible for the bacteria of a disease which is localized in one part of the body to be conveyed to

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*[*Lancet*. July 27, 1907. Abstract in *Journ. Am. Med. Assn.*, Aug. 24, 1907.

†For detail concerning mosquitoes consult the book of Dr. L. O. Howard. McClure, Phillips & Co.

‡See V. A. Moore. Infectious Diseases of Animals. 1902.

§Report on Surra. U. S. Bureau of Animal Industry. 1902.

||Carroll. *Journal American Medical Association.* May 23, 1903.

¶Guiteras. *Journal American Medical Association.* July, 9, 1904.

another part of the body, and to cause the disease in the new situation in the individual's own body. This is called *auto-infection.* It is also the case that certain bacteria remain inert when present in certain parts of the body, but cause disease when transferred to other parts. The microbes of lobar pneumonia, for instance, flourish in the mouths of a large number of people and under favoring circumstances may produce disease in the lungs or other parts, though it is not known in this case whether the infecting organism comes from the patient's own mouth or from the outside. The bacillus coli communis, which constantly inhabits the intestines, may invade other organs and exhibit pathogenic properties when the way is opened up for it by other disease processes. Persons suffering from gonorrhea frequently infect their eyes by transfering the urethral secretion.

Bodily Conditions that Predispose to Infection.—The development of an infectious disease may be favored by certain *bodily conditions* spoken of as predisposing causes. These may be general and operate in such a way as to lower the general tone of the body, as it is rather vaguely stated; or may, in addition, predispose to certain definite infection. Thus hunger, cold and exhaustion make the body more liable to the inroads of pathogenic bacteria in general; so also do anemia and chronic diseases. As an example of predisposition to specific infection is the well-known fact that those suffering from diabetes are espiecially liable to infection by the pus-producing bacteria and the bacillus tuberculosis. Prolonged anesthesia probably renders patients who have undergone operations more liable to surgical infections and to absorption of bacterial poisons. Predisposition to infection may also arise in such cases from auto-intoxication with the products of disordered metabolism of the patient's own cells. Some of the above-mentioned conditions can be imitated in laboratory experiments. Hens in a normal condition are not susceptible to the anthrax bacillus, but Pasteur succeeded in making them susceptible to this disease by artificially cooling them. Frogs, on the other hand, which also are resistant to anthrax, may be made susceptible by keeping them at an abnormally high temperature. Rats were made more susceptible to anthrax by physical exhaustion produced by making them run a treadmill and pigeons by starvation.

Abbott found "that the normal vital resistance of rabbits to infection by streptococcus pyogenes is markedly diminished through the influence of *alcohol*, when given daily to the stage of acute intoxication." This effect of alcohol was evident to a marked degree with the anthrax bacillus, but it was less noticeable for bacillus coli communis, and not observed for staphylococcus pyogenes aureus.

Climate and altitude appear to influence the liability to infection with the tubercle bacillus; for, as everyone knows, tuberculous affections occur less commonly in elevated regions than in lower and more densely populated districts.

There are probably a great many other as yet obscure conditions affecting predisposition to infection.

Age.—In general, infants are more susceptible to infections than adults, though apparently nearly exempt from the exanthematous fevers during the early weeks of life. Osteomyelitis is commoner in infants than in adults, as also is tuberculous meningitis.

Individual Predisposition.—The influence of individual predisposition is often very marked; though, as Welch says, "The fact that some individuals are attacked and others, apparently equally exposed to the danger of infection, escape, is not always due to any especial predisposition on the part of the former. It may be that the germs hit the one and miss the other, and we would have no more right to say that the former are especially predisposed than to say that those who fall in battle are predisposed to bullets and those who escape are bullet-proof." Nevertheless, as was clearly shown in the cholera epidemic in Hamburg, Germany, in 1892, many persons were found who harbored the comma bacillus in their intestines without exhibiting any symptoms of the disease. Many healthy persons have been found to harbor the diphtheria bacillus in their throats. Pneumococci are present in the mouths of many healthy individuals. From these and other examples which might be cited, it is apparent that individual resistance and individual predisfusition, due to causes as yet obscure, are important factors in infectious diseases.

It is probable that the importance of an *hereditary tendency* to certain infections, notably tuberculosis, has been overrated.

Predisposition of Different Organs and Tissues.—Certain tissues of the body are liable to attack from one kind of organism, while another tissue may be more susceptible to invasion by a different species of bacterium. The mucous membrane of the urethra is specially open to attack from the gonococcus; that of the intestines from the dysentery bacillus, the cholera spirillum and the typhoid bacillus; the lungs are more liable to attack from the tubercle bacillus than other organs; the eye may be attacked by a large number of different organisms* beside being subject to attack of a special, possibly of two or more special bacteria. Conjunctivitis has been found to be caused by the pneumococcus, the diphtheria bacillus, streptococcus, staphylococcus, gonococcus, B. coli communis, meningococcus intercellularis, several of the group of organisms to which the Friedlander's pneumococcus belongs, B. influenzæ. In addition to these organisms, a special bacterium, the Koch-Weeks bacillus, has been found to cause epidemic conjunctivitis in all lands. This is a fine bacillus much resembling that of mouse septicemia.

Race.—The influence of racial predisposition is undeniable.

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*Axenfeld in Kolle and Wassermann. Bd. III., 1903. pp. 489-575.

For example, it is known that the negro race is much less susceptible to yellow fever than the white race.

Local Conditions.—A most important influence in determining the occurrence of infections may be found in local bodily conditions. In endocarditis the lesion usually occurs along the line of closure of the heart-valves, indicating that the point subjected to the greatest friction is the part of the endocardium most liable to infection. Regions where there is passive hyperemia are more vulnerable, as is seen in hypostatic pneumonia; but on the other hand v. Bier has found that the production of passive hyperemia by artificial means tends to bring about a rapid improvement in local infections. Localities which have suffered from previous inflammation or irritation are rendered more liable to subsequent infection, as when the bladder or pelvis of the kidney containing a calculus becomes the seat of a suppurative cystitis or pyelitis.

Local conditions become of great importance in surgery. The surgeon can seldom be certain of dealing with a perfectly aseptic wound, and must rely to a large extent upon the power inherent in the fluids and tissues to prevent the development of bacteria. It is important, therefore, to keep the resisting power of the tissues at the highest possible point. Injury of the tissues disposes the part to infection; so do strangulation and necrosis. In operating, it is to be remembered that hyperemic and edematous parts are more likely to become infected; so also are anemic regions. An infarct of the lung which was originally sterile may be infected with bacteria through inhalation, and undergo suppuration or gangrene. The presence of foreign bodies in the tissues disposes to infection. Injection of the staphylococcus pyogenes aureus into a rabbit's tissues is not always followed by suppuration, but if a foreign body, like a piece of sterilized potato, be inserted at the same time, infection is much more likely to occur. When lesions are produced in the internal viscera of animals by cauterization or crushing and bacteria then injected subcutaneously or into the blood, the bacteria lodge in the lesions and multiply.*

Amount of Infectious Material.—A large number of bacteria introduced into the body simultaneously will be more likely to produce infection than a small number. This factor is of less importance with organisms whose virulence is very constant than with those of more variable virulence.

Variability in the Virulence of Bacteria.—The occurrence of an infectious disease depends very largely upon the virulence of the bacteria. Any species of pathogenic bacteria may vary in virulence at different times. In some cases the virulence is not easily lost, as with the anthrax bacillus; in others the virulence is maintained in cultures only with difficulty, as in the case of the micrococcus lanceolatus (of pneumonia) and the streptococcus pyogenes. As a rule, the virulence is likely to be diminished in old cultures. It may sometimes be preserved better in the ice-chest than at the room temperature. The virulence of the anthrax bacillus becomes diminished if it is cultivated at 42° to 43° C. Exposure to light and to oxygen tends to weaken the virulence; and also cultivation upon unfavorable media, such as those containing a small proportion of carbolic acid or certain other chemical germicides.

In laboratory work the virulence is usually maintained best by inoculating the bacteria from time to time into susceptible animals. Bacteria coming freshly from infected animals are likely to be highly virulent. The virulence may be increased by beginning with an especially sensitive animal like a very young guinea-pig, and progressively inoculating into less sensitive animals. The infection of relatively insusceptible animals may sometimes be produced by the injection of a very large dose of the bacteria. The addition of the toxic products of the bacteria, which may be obtained by using large doses of cul-

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*Cheesman and Meltzer. *Journal Experimental Medicine.* Vol. III.

tures in bouillon, makes infection more likely. Cultivation on a particular medium may maintain or increase the virulence.

Finally, the combination of two or more kinds of bacteria may produce infection when neither one would do so alone. On the other hand, it is said that the fatal effects of an inoculation of virulent anthrax bacilli into a susceptible animal may be averted if the animal be inoculated with a culture of bacillus pyocyaneus shortly afterward.

Mixed Infection.—It is not uncommon in disease to find two kinds of bacteria associated together, producing a mixed infection. In diphtheria, very frequently, the bacillus of diphtheria is found to be accompanied in the membrane by the streptococcus pyogenes. The course of the diphtheria may be modified in this manner. The term *secondary infection* is rather loosely used. It is sometimes employed to designate an infection occurring in an individual, the resisting power of whose tissues has been weakened by some chronic organic disease. Such an infection is often called a *terminal infection.* Terminal infections are very common in cases of carcinoma, chronic nephritis, arteriosclerosis, and in many other diseases.

Concerning terminal infections Osler says: "It may seem paradoxical, but there is truth in the statement that persons rarely die of the disease with which they suffer. Secondary infections, or, as we are apt to call them in hospital work, terminal infections, carry off many of the incurable cases in the wards."

The term *secondary infection* is also used for the modification of an infectious process which has been in existence for some time, by infection with a second species of bacteria. That takes place, for instance, in pulmonary tuberculosis, when the invasion of the already tuberculous lungs by the pyogenic micrococci assist in the formation of cavities. In this sense it will be seen that the term secondary infection is used as a name for a variety of mixed infection. In the secondary, mixed and terminal infections, the bacteria which enter secondarily are likely to be of the pus-producing varieties, especially the streptococcus pyogenes.

As to the mechanism which bacteria make use of in order to produce disease, according to our present knowledge, they work chiefly through the poisonous substances formed by them and deposited in the bodies of the persons suffering from the disease. The theory that bacteria have an important influence through the destruction of substances taken by them from the body of the patient for food is no longer entitled to much weight; neither are we able in most cases to account for the phenomena of disease by any mechanical action on the part of the bodies of bacteria. That such action does occasionally take place may be seen in experimental anthrax in mice, where the blood-capillaries of the liver and kidneys may be completely plugged with masses of anthrax bacilli. The diseases in which the circulating blood is swarming with bacteria are much commoner in the lower animals than in man.

Toxemia.—By toxemia is ment the absorption of poisonous bacterial products from a localized point of invasion, and their dissemination throughout the body by means of the circulation. We see typical toxemias in diphtheria and tetanus. In surgery the term *sapremia* is used to cover a similar condition of affairs when the absorption proceeds from a wound or denuded surface, as may happen in the puerperal uterus.

Septicemia.—In septicemia there is not only absorption of bacterial poisons, but an invasion by bacteria of the living tissues and the blood. The presence of large numbers of bacteria disseminated throughout the body and in the blood is less common in septicemia in man than in such diseases as anthrax in the lower animals. Typical septicemias in man are found in relapsing fever and certain cases of bubonic plague. For pyemia, see the article on Suppuration, Part IV.

The principal agencies in effecting *recovery* from infectious diseases are the destruction of the bacteria by the cells of the body (phagocytosis), the development of new substances which neutralize their action (antitoxins) and the presence or formation in the body of substances which destroy bacteria (lysins). These phenomena are discussed in the chapter on Immunity. A factor of less importance is the elimination of bacteria by the excretory organs. Investigators who have made experiments on animals disagree as to whether or not the bacteria which have been injected into the body appear in the urine before they have damaged the structure of the kidney. The extent to which the excretory organs act in eliminating bacterial toxins is not yet known. Some bacteria, as has already been stated, may, in the end, produce substances that are inimical to their own growth.

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CHAPTER VI.

BACTERIAL POISONS.*

There are now recognized three different kinds of bacterial poisons: (*a*) Ptomaines, or poisons produced by bacteria out of the medium upon which they grow; (*b*) endotoxions, or poisons contained within the bacterial cell and liberated only upon the disintegration of the cells; (*c*) toxins, or poisons liberated into the culture-medium and into the animal body by the bacteria during their growth, probably as a sort of excretory product. The ptomaines are not specific, they are not produced necessarily by pathogenic bacteria, but, as the name implies, may be developed in putrefying meat. These bodies were first studied by Brieger. They are crystalline in character. On the other hand, it is now generally accepted that in most, if not all of the infectious diseases, the principal symptoms and lesions are to be attributed to the action of either endotoxins or of toxins. Even in those cases where poisonous substances are not demonstrable in cultures or demonstrable with difficulty only, there is reason to believe that the bacteria in such cases may, nevertheless, produce poisons in the animal body.

Certain infectious diseases afford examples of poisoning by bacterial products in an extremely marked manner. In tetanus the local wound may be trifling and in itself utterly incapable of giving rise to the violent muscular spasms from which the patient suffers in consequence of the powerful poison which the tetanus bacillus forms at the point of infection. In diphtheria, although the condition in the throat may be one of severe inflammation, it is of itself insufficient to explain the profound prostration and other symptoms of general poisoning which the case manifests.

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*For a full consideration of this subject see Vaughan and Novy. The Cellular Toxins. 1902.

Bacterial poisons may be diffused through the culture-medium or they may be retained in the bodies of the bacteria. Consequently, they are classed as extracellular and intracellular. Intracellular poisons are called *endotoxins.* In cultures of the diphtheria and of the tetanus bacilli the culture-medium contains the poison, and injections of the broth in which these organisms have been grown produce these diseases just as promptly and effectually as injections of the bacteria themselves. Even when the bacteria in these cultures are entirely removed by filtration through porcelain filters, the filtrate reproduces the diseases with all their symptoms just as characteristically as the unfiltered cultures. The toxin from the diphtheria bacillus and that from the tetanus bacillus are therefore extracellular toxins, and these two are the only ones which are extracellular as far as is yet known. On the other hand, endotoxins are not liberated into the culture-medium. They are only set free by breaking up the cells, either mechanically, by grinding in a mortar, or by disintegration in some other way. The disintegration of these bacteria in the animal body is probably the way in which certain of them cause disease. Typhoid bacilli and cholera spirilla probably act in this way.

Tracy* obtained a strong toxin by disintegrating B. prodigiosus in various ways.

It seems probable that some bacteria do not produce either intra- or extracellular toxins in the strict meaning of the word. Anthrax bacilli and tubercle bacilli are of this sort.

The first bacterial poisons to be studied thoroughly were the ptomaines. The observation that the poisonous effects which follow the injection into animals of certain ptomaines derived from bacterial cultures, suggested the idea that similar ptomaines, formed by the action of bacteria in the living body, might account for the symptoms of many of the infectious diseases. The ptomaines were most readily studied because of the comparative facility with which they could be isolated in a condition of purity, where their exact chemical nature could be determined. They were found to be basic compounds derived from nitrogenous material.

**Journ. Med. Research.* Vol. XVI., No. 2, 1907. pp. 307–327.

A similar group of substances called leucomaines has been discovered, which are formed within the body by faulty metabolism and not by the action of the bacteria.

Further study demonstrated, however, that the characteristic features of the infectious diseases are not due to ptomaines, but to toxins. The term toxin, unless otherwise specially designated, applies to extracellular toxins, of which the toxin of diphtheria and that of tetanus are the types; indeed, the only representatives so far obtained are from bacteria, though there are other bodies of a similar nature found in certain plants and in snake and other venom from animals. Toxins have not been obtained yet in a pure state, and consequently their exact chemical character has not been determined; but much has been learned in regard to their physiological action, and more information in this direction is constantly being obtained by experiments. They have very marked characteristics and they do not act like ordinary poisons, but behave as if they had the power of reproduction. An ordinary poison, such as arsenic, strychnia and the like, begins to act as soon as it is absorbed—there is no period of incubation. The toxins, on the contrary, have a distinct period of incubation. If an animal is given a fatal dose of arsenic or strychnia, it succumbs within a comparatively short time; it is at most a matter of a few hours. But if an animal is injected with a fatal dose of the toxin of tetanus it takes some time, often several days, before any symptoms develop, and moreover the animal may remain alive for days afterward. In some respects the toxins resemble the physiological ferments, ptyaline, pepsin, and the like; but they differ from these in that the physiological ferments are not themselves used up in the process of fermentation, whereas the toxins are used up in the production of disease. After starch has been converted into sugar by ptyaline, the ptyaline may be recovered and used over and over again to convert more starch; but after tetanus toxin has produced tetanus in an animal it cannot be recovered, since it has become firmly united to the cells. Toxins, therefore, are very peculiar bodies, behaving like ferments in requiring considerable time to produce effects, and acting like unorganized poisons in being used up in the tissue changes which they produce. Certain substances derived from the vegetable kingdom behave in the same manner as bacterial toxins; ricin, abrin and robin are examples of these. The poisons of scorpions and snakes are also poisons which act like toxins. Other properties of toxins will be considered in connection with antitoxin.

Although, as has been stated, the toxins have not been isolated in a pure condition, they have, nevertheless, been obtained in some cases in an extremely concentrated form. Brieger and Cohn obtained a toxin from tetanus bacilli of which 0.00000005 gram killed a mouse weighing 15 grams. Roux and Yersin obtained a toxin from diphtheria bacilli of which 0.00005 gram was capable of killing a guinea-pig. These figures indicate the extremely poisonous character of these toxins. Such properties permit bacteria growing in a comparatively limited area to act upon parts of the body remote from the focus of infection.

A curious and unexplained effect of some toxins is the production of minute areas of necrosis in certain viscera, as the liver. Such "focal necroses" have been observed to be formed by the poisons of the baiclli of diphtheria, of typhoid fever, and of the Micrococcus lanceolatus of pneumonia, and following the injection of abrin and ricin.

Besides the extracellular toxins produced by the bacilli of diphtheria and of tetanus, endotoxins, have been obtained from the spirillum of cholera, the bacillus of typhoid fever, the Bacillus coli communis, the bacillus of bubonic plague, Bacillus pyocyaneus; Streptococcus pyogenes, and Staphylococcus pyogenes aureus. The extract from cultures of tubercle bacilli, called tuberculin, and that from glanders bacilli, called mallein, will be spoken of in connection with the bacteria themselves. Besides these toxic substances, lysins or bodies which disintegrate bacteria and other substances have been obtained from bacterial cultures. Thus pyocyanase disintegrates many kinds of bacteria (see p.—). Some bacteria seem to contain lysins which disintegrate the bacteria themselves in which they are produced—so-called autolysins. Vaughan* has succeeded in cultivating anthrax bacilli, colon bacilli, and other bacteria on large surfaces of solid media, so as to secure quantities of the bacterial cells sufficient for extensive chemical tests. The toxin of the colon bacillus proved to be a very stable substance, and resistant to heat. Most toxins become inactive at comparatively low temperatures (60° to 70° C.).

The endotoxin of plague † is destroyed by 70° C.; that of dysentery by 80° C., that of typhoid by 127° C.

Other physiological properties of the toxins will be brought out in connection with the discussion of immunity.

There is good reason on both clinical and experimental grounds to believe that toxic substances are formed by the Micrococcus lanceolatus of pneumonia.

In connection with bacterial poisons another class of bodies may be conveniently described; these are *agglutinins*, *lysins* and *precipitins.*

Agglutinins.—The blood-serum of human beings as well as of animals suffering from certain diseases has the power of causing the bacteria of the disease from which the individual has recovered to clump into larger or smaller masses in liquid cultures to which the serum is added. The same phenomenon is observed in the serum of animals injected with repeated doses of cultures. This is due to certain substances called agglutinins. The reaction, while it is more or less specific, is not as strictly so as was formerly thought, for it has been found that a given agglutinin may cause clumping of a group of nearly related bacteria; such an agglutinin is called a group agglutinin; and, moreover, under certain circumstances the

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**Journal American Medical Association.* September 3, 1904.

† Besredka. *Bull. de l'Inst. Past.* No. 13, Vol. IV., July 15, 1906. p. 587.

bacteria fail to clump in the blood of patients suffering from a given disease. Again, in some cases the serum in a certain disease will clump bacteria that are not concerned in the production of the disease. Even normal serum will sometimes clump bacteria. The serum from a given disease is said to be homologous with the bacteria causing the disease and heterologous from other bacteria, and the bacteria are said to clump or not to clump with homologous sera as the case may be. Bacteria are also homologous or heterologous in the same sense toward sera.

Park and Collins,* in summing up their experience in the study of agglutinins among other things, state that the injection of animals with bacteria causes the production of agglutinins which are specific for the organism used for injection, but in addition to this it also causes the production of agglutinins which agglutinate bacteria other than those injected; not only closely allied bacteria, but those which differ widely in their biochemical as well as in their specific agglutinating characteristics. The injection therefore forms specific, group and heterologous agglutinins. Of these there is at first more specific agglutinin than of the others. Further injection may cause a more or less rapid diminution of the specific agglutinin along with a less rapid diminution of the others, so that the relative amounts of the different agglutinins may vary. The heterologous agglutinins may be absorbed by injecting the immune animal with various bacteria other than the one used for the immunization so as to free the serum from all but the specific agglutinin so that it will clump only the bacteria which were first used for the immunization. Group agglutinins may also be absorbed by injecting the immune animal with bacteria nearly allied to that used for immunization. Thus the typhoid bacillus will absorb many group glutinins for the colon bacillus. The injection of bouillon into rabbits will frequently produce agglutinins for several colon types; this is probably due to products of bacterial growth which has taken place in the meat extract before its preparation as a culture-medium. Agglutinins and lysins bear no relation to one another.

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* *Journ. Med. Research.* XII., pp. 491–507. 1904.

Park* points out that the majority of bacteria do not develop sufficient agglutinin in the course of the disease which they cause to be detected, as in the case of tubercle, influenza and diphtheria bacilli. In regard to group agglutinins he shows that bacteria may produce such a large amount of agglutinin in the course of the infection that they not only agglutinate themselves with the blood serum from the patient, but that widely different bacteria may also agglutinate with the serum. Thus he found that an animal injected with staphylococcus agglutinated the typhoid fever bacillus in the proportion of 1–160 while, before the serum from this animal agglutinated the typhoid bacillus in the proportion of only 1–10. Park gives further examples of the same sort. But on the other hand, he points out that in practice such conditions will not be met with, and it may be regarded as certain if the typhoid reaction is obtained with the serum from a patient in the proportion of 1–50 in two hours at room temperature that the patient is suffering from infection with a member of the typhoid-colon group is present, probably the typhoid bacillus.

Park† also found that bacteria cultivated on homologous sera‡ lose their property of agglutination with the kind of serum used as a culture medium, but recover this when cultivated upon the ordinary culture-media. Weil§ obtained a culture of typhoid bacillus from an abscess in the thyroid of a typhoid convalescent which did not agglutinate with the patient's serum nor with other homologous sera.

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*Park. *Journ. Infectious Diseases.* Supplement No. 2, 1906. pp. 1–9.

†W. H. Park. Proceedings of the New York Pathological Society. Vol. IV., Nos. 1 and 2. February and March, 1905.

‡See p. 191.

§Weil. Proceedings of the New York Pathological Society. Vol. IV., Nos. 1 and 2. February and March, 1905.

In spite of the fact that agglutination has been found to take place spontaneously in cultures, it is, nevertheless, in the main a specific reaction, and is employed as an aid in the diagnosis of typhoid fever, where it is spoken of as the Widal or Gruber-Widal test. Under proper precaution it is valuable in this special case, and will be referred to again in connection with the description of the typhoid bacillus.

Other bacteria which agglutinate with the homologous sera are: Spirillum of cholera, B. pyocyaneus, B. proteus, B. coli communis, Micrococcus melitensis, B. mallei, B. tuberculosis, Diplococcus pneumoniæ, B. pestis bubonicæ, and B. dysenteriæ. Trypanosomes also agglutinate with homologous sera.

Lysins.—There are certain substances found normally present or produced artifically in the blood which have the property of breaking up foreign red blood-cells introduced into the circulation or into the blood-serum outside the body. This is not only true of red blood-cells, but certain bacteria also become broken up when introduced into the blood of certain animals. This process is spoken of as cytolysis, and when occurring in red blood-cells, is called hemolysis; in bacteria, bacteriolysis. The substances causing cytolysis are called lysins. Lysins have also been obtained from cultures of bacteria. Thus Besredka* obtained a cytolysin, streptolysin by filtering virulent cultures of streptococcus strongly hemolytic for the red corpuscles of many animals. Reudiger† corroborated Besredka's work and extended his observations upon the nature of streptolysin. As already stated, lysins for certain foreign cells are normally present in the serum of certain animals; thus, human red blood-cells are disintegrated by sheep's serum, rabbit's blood-serum disintegrates anthrax bacilli, andnumerous other examples exist of lysin normally present in blood-serum. But whether normally present or not, specific lysins, like specific agglutinins, are made to appear in the blood-serum of animals by injecting these with several doses of suspensions of cells. Hemolysin results from injecting an animal with the red blood-cells from another animal, bacteriolysin from the injection of bacteria. The serum of the blood of animals injected in this way is called immune serum. Thus an animal injected with B. typholococcus furnishes typhoid-immune serum; an animal injected with rabbit's blood-corpuscles furnishes rabbit-corpuscle-immune serum. Hemolysins and bacteriolysins are quite sharply specific. A rabbit injected with a suspension of red corpuscles from the blood of a guinea-pig furnishes hemolysins which destroys guinea-pigs' red cells. A guinea-pig injected into the peritoneal cavity with repeated small doses of the cholera spirillum furnishes a peritoneal fluid containing a bacteriolysin specific for the cholera spirillum. Still, group lysins, like group agglutinins, are also found, for while lysis takes place more promptly and in smaller amounts with the cells of the same species of animal or with the same kind of bacteria with which the animal furnishing the cytolytic serum has been injected, it also occurs in a less marked degree with cells from nearly related animals or with nearly similar bacteria.*

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* Ann. de l'Inst. Past. 1901, No. 15, p. 880.

† *Jour. Inf. Dis.* Vol. IV., No. 2, April 10, 1907. pp. 277–281.

The same serum is not equally potent for different bacteria, and the serum from different animals of the same or of different species varies in bactericidal potency for the same organism. The chemical reaction of the serum seems to exert some effect upon this quality of the serum. The more alkaline the serum the more potent is its action apparently, and consequently the venous blood has been found sometimes to furnish more potent serum than the arterial blood from the same animal.

Serum loses in bactericidal potency on standing after being drawn from the animal; and the higher the temperature to which the serum is exposed the more rapid the loss of potency. It may remain potent for several days or for even a week or more in the refrigerator, but if kept at body temperature it generally loses all bactericidal properties in three or four hours. Heating at 55° or 56° C. for ten or fifteen minutes also robs the serum of its bactericidal power, or rather this treatment of immune serum suspends its bactericidal power, which is restored by the addition of a small amount of fresh serum. This suspension of bactericidal power by heating at 55° or 56° C. is called inactivating the serum. Inactivated serum to which fresh serum is added, and which has had its bacteriolytic properties restored in this way, is termed reactivated serum. There are probably many circumstances which influence the bacteriolytic property of the serum. The chemical reaction of the serum has been found to influence the bactericidal power, and doubtless there are other as yet obscure circumstances which raise or lower this power of the serum. The nature of bacteriolysins will be found discussed at some length below, and it would not seem at all improbable that there may be more of these at one time than at another present in the blood-serum. Indeed, the production of bacteriolysis in serum of the living animal seems to be easily influenced one way or another, and it would not be unreasonable to regard them as varying from time to time under even slightly changing conditions of the body.

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*Further reference: Pribram. Kolle and Wassermann. Ergänzungsband. 1906. pp. 291–346.

Trommsdorf* noticed that human sera derived from normal individuals as well as from those suffering from various diseases vary greatly in bacteriolytic power. Petterson† found the same thing with chickens.

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*Richard Trommsdorf. Ueber den Alexingchalt novmaler und pathologischer mauschlicher Blutsera. *Centralbl. f. Bakt.*, etc. abt 1. Orig. Bd. 32, No. 6, pp. 436–449. 1902.

†J. Morgenroth and H. Sachs. Ueber die Completierbarkeit der amboceptoren. *Berl. klin. Woch.* Jahrg. 39, No. 27, pp. 631–633.

Morgenroth and Sachs* found great variation in cytolytic power in serum of various sorts. Thus the serum from a horse at one drawing was hemolytic for rabbits' corpuscles but not for those of guinea-pigs; three days later the serum from the same horse was strongly hemolytic for guinea-pigs' corpuscles, but only very slightly for rabbits' corpuscles; twenty-three days later the serum from this horse was not hemolytic for guinea-pigs' corpuscles, but strongly hemolytic for rabbits' corpuscles.

It is therefore evident that the cytolytic power of serum is very variable. Not only does the blood from different individuals of the same species differ in this respect, but the serum from the same individual differs from time to time. This is probably the case with all animals.

The peculiar behavior of immune sera on dilution will be described under immunity.

Precipitins.—Precipitins are bodies which develop in the serum of animals which have been given subcutaneous injections of albuminous substances, and which, added to solutions of the albumin with which the animals have been injected, cause these to become cloudy and finally form a precipitate. Thus a rabbit's blood-serum may be sensitized by injections of hen's egg-albumen, and the rabbit's blood-serum will then precipitate hen's egg-albumen. It may, however, imperfectly precipitate albumen from the egg of a species closely allied to the hen.

Again, a rabbit injected a few times at intervals of a day or two with human blood-serum furnishes serum which even in small quantities causes precipitation even in a weak solution of human blood-serum, such as may be obtained from old dried blood-spots.

The effect produced by the injection of foreign albumin

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*Alfred Petterson. Ueber die natürliche milzbrandimmunität des hundes und des huhns. *Cent. f. bakt.*, abt. 1, Orig., bd. 33, no. 8, p. 613–626. Jena, Apr. 4, 1903.

is regarded as analogous to that produced by the injection of bacteria, and consequently the serum of the animal injected with albumin is spoken of as immune serum in the same way as the serum from an animal injected with bacteria is so designated. The serum from the rabbit in the last example is human serum immune-rabbit-serum.

The term homōlogous is employed to denote the relation between the immune serum and the albumin used in its production. Thus, in the example just given, the rabbit's serum and human serum would be called homologous the one with the other.

The reaction is very sensitive, the immune serum causing clouding in solutions containing very small traces of the homologous serum. Rabbits are usually employed for the production of immune sera for the precipitation reaction, and seem specially adapted to the purpose.

The reaction is of great value in determining the kind of blood in any doubtful case, and is applied practically in forensic medicine to determine the character of suspicious bloodstains.

In the precipitin reaction, as this is called, there is group precipitation, it is true. Thus, human and monkey's sera react with the same precipitin and dog's and wolf's sera respond to the same precipitin. Bacterial precipitins have also been obtained by injecting animals with bacteria. In this case filtrates obtained by filtering bacterial cultures or suspensions through porcelain filters give a cloud, with ultimate precipitation, when treated with a drop of homologous serum.*

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*Norris. The Bacterial Precipitins. *Journal of Infectious Diseases.* Vol. I., p. 463. 1904

CHAPTER VI.

IMMUNITY.

Studies in immunity have led to remarkably uniform results in so far as the facts are concerned and there is great unanimity in regard to the actual observations, both of the processes which take place spontaneously in nature, as well as of those which follow in the intentional experiments. There is, however, great difference of opinion upon the interpretation of these phenomena, and several opposing theories have been advanced in regard to the mechanism concerned, each theory finding very eminent supporters.

In view of these facts, it is necessary, in discussing immunity, to give a definition covering its broadened application, to cite the observations which have been recorded and to present the prevalent explanations offered by the various authorities.

It is scarcely necessary to go into a discussion of the various theories which have been advanced from time to time, but which have now been abandoned.

Immunity, as formerly studied, embraced only considerations of the insusceptibility of individuals or of races to an attack of a given infectious disease. But the modern conception is broader than this, and it is no longer confined to immunity proper, but extends to certain other processes which have been found to bear a close resemblance, in certain respects, to immunity, and to be governed by laws very similar to those which govern the latter.

Immunity at present is made to include, besides insusceptibility to infection—*i. e.*, resistance to the invasion of living bacteria—the processes concerned in the forming of the antibodies, antitoxins proper, antiagglutinins, antilysins and antiprecipitins.

Resistance to Infection.—Immunity from infectious diseases is either natural or acquired, active or passive.

By natural immunity is meant the inherited power possessed by certain races or individuals, independently of size, habits or surroundings, to resist infection to which other races or individuals are subject. This is illustrated by many examples. Rats are ordinarily insusceptible to anthrax, whereas mice, guinea-pigs, sheep, cattle—in short, most animals—are very susceptible. Mice are not susceptible to diphtheria poison on inoculation, while horses, sheep, goats, guinea-pigs and many other animals are very susceptible. Even very nearly related species or varieties often show difference in susceptibility. House-mice are susceptible to mouse septicemia, European field-mice are not. On the other hand, with glanders, house-mice and white mice are less susceptible than European field-mice are to this disease. Negroes are insusceptible to certain diseases to which the white race is very subject, and also the reverse.

Instances of individual immunity are seen in every epidemic, where persons escape when they are under the same conditions as those who have contracted the disease. Instances also occur in which nurses and others thrown with cases of highly infectious diseases escape. Some of these cases, it is true, belong more properly to the category of immunity acquired by recovery from an attack, since nurses and others thrown in contact with an infectious case may suffer a very mild attack or even, probably become immunized without showing any symptoms of disease. Nurses and physicians have been found with diphtheria bacilli in their throats and yet not showing any symptoms. Of course, these persons may have had natural immunity, but it is equally possible that they may have become gradually immunized.

Furthermore, as just stated, immunity probably also results from intimate contact with diseased individuals.

Acquired immunity follows recovery from a spontaneous attack of certain diseases, and it also results from intentional inoculation. Immunity after recovery is so familiar that no illustration is necessary. But not every infectious disease leaves immunity behind; many, on the contrary, are followed by no increased resistance, or if there is increased resistance, it is very transitory; some even tend to increase susceptibility. Examples of infectious diseases leaving no lasting immunity, and even in some cases apparent increased susceptibility, are erysipelas, diphtheria, influenza, pneumonia, gonorrhea. In those cases in which immunity follows recovery from a spontaneous attack of an infectious disease the process is probably the same as that which takes place after intentional inoculation.

Immunity acquired after intentional inoculation is produced either by inoculation with material which produces a mild type of disease, as in vaccination for small-pox; or by giving at short intervals inoculations of the disease-producing virus of graded strength, beginning with greatly attenuated material, and followed with stronger and stronger material, as in inoculation for hydrophobia and anthrax; or by injections of larger and larger amounts of bacterial poison, as in the production of antitoxin for diphtheria and tetanus; or, finally, by the injection of the antitoxin formed in the blood by the method last mentioned, as in the treatment of diphtheria and tetanus, for this, after all, is, in a way, a process of immunization.

Small-pox and Vaccination.—The origin of vaccination against small-pox with the virus of cow-pox has been described in the historical sketch. The nature of the protection furnished by this virus has been the subject of much controversy. The opinion of the present day inclines to regarding vaccinia as small-pox which has been modified by passage through a relatively insusceptible animal. Councilmann* and his co-workers regard cow-pox and small-pox as identical. But Law, Salmon and Smith† regard the two diseases as different.

This question cannot be settled with certainty until the organisms causing small-pox and vaccinia have been isolated in pure culture. Their identity and mode of action may then be determined.

Small-pox has been inoculated into calves and passed through other calves in succession, producing finally an eruption indistinguishable from cow-pox. Not only does recovery from spontaneous or artificial cow-pox protect against small-pox, but it has been shown that recovery from small-pox protects against cow-pox.

Attenuated Virus and Cultures in Which the Bacteria are Killed.—Pasteur conceived the idea of attenuating the virulence of the bacilli of fowl-cholera by prolonged exposure to the air. He made use of the virus thus attenuated as a vaccine against the disease.

A nearly similar principle was shortly afterward applied by him to the preparation of a vaccine against anthrax. When anthrax bacilli were cultivated at a temperature of 43° C., Pasteur obtained bacilli of very slight virulence. Such bacilli did not produce death when inoculated into animals that were ordinarily susceptible. Yet animals that were vaccinated with this virus were able afterward to resist inoculation with fully virulent anthrax bacilli. (See Bacillus anthracis, Part, IV).

Successful methods of inoculation have been found for *rinderpest*, an infectious disease of cattle in which the digestive organs are mainly involved. There are three ways which are efficacious:‡ (1) Injection of bile from an animal recently dead of rinderpest; (2) inoculation of glycerinated bile, followed by injection of pure bile or virulent blood; (3) by the simultaneous injection of strong standarized serum and virulent blood.

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* *Journ. Med. Research.* VII. No. 1.

†Diseases of Cattle. Dept. Agr. Pub. 1904. p. 237 and p. 426.

‡Diseases of Cattle. Dept. Agricult. Publication. 1904. pp. 380–381.

In the case of erysipelas of swine (French, *rouget;* German, *Schweinerothlauf*) Pasteur secured bacilli of diminished virulence by injecting virulent bacilli into relatively insusceptible animals. The animal used was the rabbit. The bacilli were passed through several rabbits in succession. Cultures taken from the last of the series produced a milder form of the disease and amount of immunity to a certain degree. The practical value of this inoculation is not settled.

In still another disease, black-leg of cattle or symptomatic anthrax (French, *charbon symptomatique;* German, *Rauschbrand*), an attenuated virus is secured by the use of heat. The pulp from the infected muscle of a diseased animal, containing the bacilli, is squeezed from it and heated to a temperature of 95° to 99° C. for six hours. The dried material mixed with water constitutes the vaccine. The Department of Agriculture of the United States now furnishes this vaccine free to farmers. The results of this method are said to be very satisfactory.* In the human disease, bubonic plague, a nearly similar procedure has been proposed by Haffkine. To protect against plague, cultures of plague bacilli are used which have been previously sterilized by heat, with carbolic acid added as a preservative. (See section on Bubonic Plague, Part IV.) In the preparation of these "vaccines" it is of the utmost importance to use pure cultures, and to be sure that the cultures are dead. It is not enough to subject them to heating or to the action of other germicidal agents as recommended in books, but they should in all cases be tested with cultures to determine whether all the bacteria have been certainly killed.

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*See Annual Reports, Bureau of Animal Industry, U. S. Department of Agriculture.

Inoculation Against Rabies or Hydrophobia.[*]—The immunity produced in this case probably depends upon principles similar to those underlying the examples related on the preceding pages. But this question cannot be regarded as settled until the organism of rabies has been isolated and cultivated. Attempts to discover this organism have, as yet, been futile, though certain minute bodies, bodies of Negri, have been observed within ganglion-cells of the central nervous system from cases of rabies, and it has been claimed that they are protozoa and the cause of the disease. Whether this is true or not, Negri bodies make a most valuable means of rapid diagnosis.

Frothingham[†] regards the presence of the Negri bodies as sufficient for diagnostic purposes without animal inoculation. If these are not found in smear preparations from Ammon's horn, they must be further sought in sections from this region or in the cerebellum or in the Gasserian ganglion. If all these tests are negative, and there are no lesions in the Gasserian ganglion, then animal inoculation may be resorted to merely to allay uneasines of the patient.

Davis[‡] calls attention to the fact that the Negri bodies are sometimes absent in undoubted cases of hydrophobia. Such cases are very few, and these may be diminished by improved technic. For fixing Zenker's fluid and corrosive sublimate are available, formalin is not. No special stain is required; any ordinary nuclear stain will answer as well as Koch, Unna or other modifications of Romanowsky. Davis advises that the suspected animal should be kept alive in a cage for observation. If it has hydrophobia it will die in a few days. The brain should be removed after death and examined for Negri bodies and if these are found any person who has been bitten should be given the Pasteur treatment. If the bodies are not found animals should be inoculated. He makes the remark that whatever the nature of these bodies, whether they are parasites or degenerated cells, they are characteristic of hydrophobia, but are not identical with the virus. For the hypocampus where they are found in greatest abundance is not more virulent than other parts of the brain in which they may not be found. The stain when used is diluted in the proportion of one drop of the stain to 1 c.c. of distilled water which has been rendered alkaline by the addition of one drop of a 1 per cent. solution of potassium carbonate to 10 c.c. of water. This diluted stain is poured over the dried specimen and allowed to remain for from one-half to three hours or even longer. The preparation is then washed in running tap water for one to three minutes, and dried with filter-paper. The cytoplasm of the Negri bodies takes a blue color, the central bacillus and chromatoid granules stain blue-red or azure. The tinge depends upon the thickness of the specimen. The cytoplasm of the nerve-cells stains blue also, but the nuclei of the nerve-cells stain red, the nucleoli dull blue.

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[*] For a review of works on rabies see Remlinger. *Bulletin de l'Institut Pasteur.* II., Nos. 19 and 20. 1904.

[†] *Journ Med. Research.* XIV. 1905. pp. 471–489.

[‡] *Journ A. M. A.* July 14, 1906. pp. 87–89.

For diagnostic purposes the method may be shortened as follows: Methyl alcohol, five minutes; equal parts of the Giemsa solution and distilled water, ten minutes.

In Mallory's method the smears are fixed in Zenker's fluid for one-half hour; rinsed in tap water; one-quarter hour, 95 per cent. alcohol and iodin; 95 per cent. alcohol, one-half hour; absolute alcohol, one-half hour; eosin solution twenty minutes; rinsed in tap water; methyline blue solution, fifteen minutes; 95 per cent. alcohol, one to five minutes; dry with filter-paper.

Anna Wessel Williams and Lowden* recommend the use of smear preparations of the brain for examination of the Negri bodies for the reason that the bodies appear more characteristic in such preparations than in preparations made in other ways. The smears are prepared as follows:

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*Williams and Lowden. *Journ. Infectious Diseases.* Vol. III., 1906. pp 452–483.

Very thin slices one each from near the fissure of Rolando, from Ammon's horn, and from the cerebellum are cut at right angles to the surface taking the gray matter. These are spread upon scrupously clean slides, and rubbed over in one direction gently with cover-glasses. The smears are allowed to dry in the air, and stained by Giemsa's or by Mallory's methods. Giemsa's stain as last recommended consists of: Azure II 0.8; eosin 3.0 gr.; glycerin (chem. pure) 250 c.c.; methyl alcohol (chem. pure) 250 c.c. Both glycerin and alcohol are heated at 60° C. The dyes are put into the alcohol and the glycerin is added slowly, stirring. The mixture is allowed to stand at room temperature over night, and is ready for use after filtering.

Luzzani* reports the results of himself and those of others in regard to the presence of Negri bodies. This report shows that the bodies were not found in 9 out of 296 cases in which hydrophobia diagnosis was confirmed by inoculation. In the other cases it was found either in sections or smear preparations.

Marie,† contrary to the usual experience, found that the blood of animals affected with rabies may be virulent under circumstances not yet determined.

Di Vestea‡ found that the virus of rabies, which is well preserved by glycerin in the nerve-tissue, is not so preserved when filtered through unglazed porcelain. In fact, the virus after filtration is in all respects much more labile than when not filtered. Di Vestea comes to the conclusion from this observation that only the young forms of the parasite pass through the filter. Moreover, that the results with glycerin as a preservative are in favor of the idea that rabies virus is intracellular, probably since the glycerin kills the young parasites which have not yet entered the brain cells, but not those contained in the brain cells.

Remlinger§ found that centrifugalizing rabies virus for one

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*Arch. per le sc. med. T. XXVIII., No. 34, pp. 521–540. Abstract in *Bulletin de l'Inst. Pasteur.* Vol. III., No. 7. April 15, 1905. pp. 297–298.

†Compt. Rendus Soc. Biologie, T. LVIII. 25 Mars, 1905, p. 544. Abstract in *Bul. de l'Inst. Past.* Vol. III., No. 11. June 15, 1905. p. 461.

‡Comm. Ac. Médic. Pise, 24 Jan., 1905. p. 2. Abst. *Bul. de l'Inst. Pasteur.* T. III., No. 8. 30 Avril, 1905. p. 338.

§Compt. Rendus Soc. Biologie. T. LVIII., 7 Janv., 1905. p. 27. *Abst. Med. de l'Inst. Pasteur.* T. III., No. 7. 15 Avril, 1905. p. 301.

hour with 1100 revolutions a minute, using the virus diluted 1 to 50 and 1 to 100 deprives the virus of its virulence. He also found that the virus traveled along the nerves and not the lymphatics in the rare cases of successful inoculation of animals into a pathologically hypertrophied ganglion.*

Tizzoni and Bongiovanni† found that radium rays not only destroy the virulence of rabies virus *in vitro*, but also counteract the effects of the virus in animals inoculated and exposed to the rays, and furthermore the rays transform the virus into an excellent antirabic virus. Still more remarkable is their statement that radium rays cure the disease after symptoms have developed.

Pasteur discovered that rabies could be produced in animals by inoculation under the dura mater with portions of the spinal cord of a dog suffering from hydrophobia. He also found that successive passages through a series of rabbits greatly increase the virulence of the virus, as indicated by a much shorter period of incubation after inoculation. The first rabbit of the series inoculated with the "street" rabies virus—*i. e.*, from a spontaneous case in a dog—dies in about two weeks, and each succeeding rabbit dies in a shorter and shorter time until ultimately the incubation period is reduced to six or seven days. Beyond this the strength of the virus cannot be increased, and is called "*virus fixe*," or the fixed virus. Pasteur found, moreover, that the cord of the rabbit which has attained this degree of virulence is attenuated by various agencies, notably by drying, and that animals injected with this attenuated virus can withstand inoculations of more potent virus. By drying for various lengths of time a series of "vaccines" of exactly graded potency is obtained, and starting with the vaccine of least potency an animal can be inoculated with increasingly potent virus until it will withstand inoculations of the *virus fixe* itself.

**Bull. de l'Inst. Past.* Vol. IV., 1906 p. 448.

†Acad. d. sciences de Bologne. Avril, 1905. p. 6. Abst. *Bul. de l'Inst. Pasteur.* T. III., No. 13. 15 Juillet, 1905. p. 582.

Omitting all but the chief details, the vaccines against hydrophobia are prepared as follows:*

The cord of a rabbit dead from the subdural inoculation of *virus fixe* is hung up in a long glass cylinder in the bottom of which is placed potassium hydrate. The cylinder is placed in a cool place, and every day small bits of the cord are cut off and preserved in a vial of glycerin. The virus which has been dried for thirteen or fourteen days is no longer capable of producing hydrophobia in rabbits, but an animal inoculated with it can withstand inoculation with the cord dried for a shorter time, and after inoculation with the latter withstands inoculations with cord dried for a still shorter time.

In human beings it is customary to start with the virus which has been dried for nine or ten days, injecting subcutaneously emulsions of the dried cord, and, if time permits, to give an inoculation every day with virus dried for a shorter and shorter time. As the incubation period for human beings bitten by a mad dog is quite long,—about six or eight weeks,—there is ample time to run in all the inoculations if these are begun promptly, and if in this way the individual is made to withstand the *virus fixe*, it is more than probable that the weaker virus from the dog will not be able to cause any disease.

Where much time has elapsed after the bite of the mad dog, it is sometimes the practice to give three or more injections of increasing strength every day.

These inoculations against hydrophobia have proved to be most valuable, as the large number of reports from various Pasteur institutes in various parts of the world abundantly prove. According to statistics, collected by Ravenel, based on many thousands of cases, the mortality from rabies in those so treated is less than 1 per cent.†

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* Valuable information in regard to the preparation and mode of using hydrophobia virus was contributed in a personal letter to Dr. Williams by Dr. Jas. G. Cummings, Pasteur Institute, University of Michigan.

†See Ravenel and McCarthy. *University of Pennsylvania Medical Bulletin.* June, 1901. Also editorial in *Phildelphia Medical Journal.* March 14, 1903. And Mohler. Twelfth Annual Report of the United States Bureau of Animal Industry.

In all cases where a human being has been bitten by a dog that is suspected of having hydrophobia, the individual should submit himself to the Pasteur treatment as soon as possible, if it is feasible to do so. Since this treatment, if done by competent hands, insures the person who has been bitten against the danger of the development of the disease.

In order to find out whether the dog has hydrophobia, the animal may either be put up and observed to see whether characteristic symptoms develop, or it may be immediately killed. In the latter case the brain and cord should be examined for the presence of the Negri bodies already alluded to, and the intervertebral ganglia for the presence of round-cell infiltration which is often marked. Rabbits or guinea-pigs should also be inoculated under the dura. As stated in the beginning, it is safer not to wait for the result of the examination, but to subject the person, who is bitten, to the Pasteur treatment in advance of this, unless it is improbable that the dog is mad. The examination consists in looking for the Negri bodies and in subdural inoculation of rabbits. If the cord of the dog can be obtained, the intervertebral ganglia will show round-cell infiltration. All other ganglia may also show this change. The plexiform and Gasserian ganglia are convenient for examination.

Great care must be taken that the operator may not accidentally infect himself.

Antitoxins.—Antitoxins have as yet been produced for the extra-cellular toxins only, and only those diseases which are caused by bacteria which form extra-cellular toxins have been successfully combated in this way. Antiendotoxins, on the other hand, have not yet been satisfactorily produced. Buxton* found that rabbits immunized with typhoid bacilli do not become appreciably more resistant than normal rabbits to the endotoxin of the typhoid bacilli.

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* *Journ. Med. Research.* V. XVI., No. 2, May, 1907. p. 264.

The methods used in the production of antitoxins were introduced by Behring, who found that by injecting susceptible animals with increasing amounts of extra-cellular toxin he produced in the blood-serum of the injected animal certain changes which made the serum capable of counteracting the same toxin when injected into other animals. Thus, a sheep treated with increasing doses of diphtheria toxin, beginning with very small amounts, furnishes blood-serum which protects other sheep or guinea-pigs or other susceptible animals from fatal doses of diphtheria toxin. In practice, the bacilli are cultivated in bouillon. The cultures are freed from all living bacilli by filtration. The liquid filtrate contains the toxin. This filtrate is injected into healthy susceptible animals, in increasing doses. Usually the horse is used, since large quantities of blood can be drawn from this animal on account of its size, and, moreover, the horse is very susceptible. Insusceptible animals cannot be made to yield antitoxin, at least of any appreciable strength. Eventually enormous doses of toxin are given, and the animal acquires a high degree of immunity. The blood of the animal is withdrawn, taking care to avoid contamination, and the serum allowed to separate in the refrigerator. The serum of the blood is drawn off and constitutes the antitoxin. The use of antitoxin has been eminently successful and revolutionized the treatment of diphtheria; and it has given complete success as a prophylactic in tetanus with an antitoxin prepared by injecting horses with increasing amounts of tetanus toxin. (See the description of the bacteria of these diseases.)

Ehrlich discovered that the vegetable toxins, abrin and ricin, behave in a manner very similar to soluble bacterial poisons when injected into animals, and that by their injection an immunity for the same poisons may be secured. Ehrlich also found that the milk of animals which had been immunized with increasing doses of abrin and ricin confers immunity upon sucklings.

There is little, if any, analogy, on the other hand, between the tolerance acquired by gradual dosage from bacterial and other toxins and that which victims of the morphine and cocaine habits have for immense doses of these drugs, for no bodies resembling antitoxins are obtained from animals that have been accustomed to such drugs.

The gradual injection of animals with bacteria which produce endotoxins, B. typhosus, B. choleræ Asiaticæ, B. coli communis and others, does not produce antitoxin. It sometimes seems to have no effect, at others it seems to increase the bacteriolytic power of the blood for the bacteria with which the animal is injected. Frequently it has a most peculiar effect. This peculiarity was first shown by Loeffler and Abel* in experiments upon guinea-pigs injected with colon bacilli at the same time given injections of colon-immune dog's serum. The very unexpected result was obtained that those guinea-pigs which received smaller doses of the immune serum were protected, whereas those injected with larger doses were not protected. Similarly, Neisser and Wechsberg found in test-tube experiments with cultures that the serum from the injected animal sometimes kills the kind of bacteria used for injection when it is diluted but not when it is undiluted. This action is so paradoxical that it may well be stated in other words for emphasis. The normal blood-serum of uninoculated animals, as has been previously stated, is bactericidal for many bacteria. Now, if an animal furnishing normally highly bactericidal serum is injected with an endotoxic bacterium, its blood-serum often fails to kill the kind of bacterium with which it is injected, unless the serum is greatly diluted. It still retains its power to kill other kinds of bacteria.† The peculiar behavior of this so-called immune serum is called the Neisser-Wechsberg* phenomenon. The theoretical explanation of this phenomenon will be discussed below under Ehrlich's side-chain theory.

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*Loeffler, F., and Abel, R. Ueber die spezifischen Eigenschaften der Schutzkörper im Blute typhus- und coli-immuner Tiere. Cent. f. Bakt., Abt. 1, Bd. 19, No. 2, 3, p. 51–70. Jena, Jan. 23, 1896.

†B. H. Buxton. Bacteriolytic Power of Immune Serum and the Theory of Complement Diversion. *Journal of Medical Research*, V. XIII., No. 5. (Whole No. 90, N. S. V. S.) p. 431–485. Boston, Aug. 1905.

Buxton† reports that the immunization of a rabbit with typhoid cultures does not enable this animal to dispose of a sublethal dose of the bacilli any more quickly than the normal rabbit and that such injections do not produce any appreciable increase of resistance to the endotoxin of the typhoid fever bacillus.

Analogous to the hypersensibility to infection sometimes noted on the injection of animals with repeated doses of bacteria is the phenomenon of *anaphylaxis* seen in animals injected with repeated dose of foreign serum. Animals injected with egg albumin or with blood-serum derived from some other species withstand an amount of the albumin at the first injection which kills them on subsequent injection. Rosenau and Anderson‡ have found that the reaction of the animals in this case is specific. That is, guinea-pigs sensitized with horse serum are very sensitive to a second injection with horse serum, but very slightly susceptible to injection with the serum from other animals such as rabbit, cat, dog, hog, sheep, chicken or man. Furthermore, a guinea-pig may be rendered sensitive to three different kinds of proteids at once by injecting the proteids either all simultaneously or all separately.

Active and Passive Immunity.—The kind of immunity which results from the injection of substances from immunized animals is called "passive immunity." Diphtheria and tetanus antitoxins produce passive immunity. "Active im-

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*Max Neisser and Friedrich Wechsberg. Ueber die Wirkungsart Bactericider Sera. *Münchner Med. Woch.* Jahrg. 48, No. 13, p. 697–700. Münch. Apr. 30, 1901.

†*Journ. Med Research*, V. XVI., No. 2, May, 1907. pp. 251–274.

‡Hyg. Lab. Bul. No. 29 and Bul. No. 36. U. S. Public Health and Marine Hosp. Serv. April, 1906 and 1907 resp. Also *Journ. Infec. Dis.* Vol. IV., 1907. pp. 552–557.

munity," on the other hand, is that which results in some cases:

(1) From a spontaneous attack of an infectious disease; (2) from an attack excited artificially through inoculation with small doses of virulent cultures; (3) from the administration of large doses of attenuated cultures; (4) from the injection of bacterial products (toxins) freed from the bacteria themselves. Pasteur's methods of protective inoculation for anthrax and other diseases, and Haffkine's injections for bubonic plague, the gradual injection of cultures of diphtheria or of tetanus bacilli into animals, produce active immunity. Active immunity is usually more enduring than passive immunity. But passive immunity, resulting, as it does, from the direct introduction of antitoxin, is brought about more quickly than active immunity.

THEORIES OF IMMUNITY.

Phagocytosis.*—Metchnikoff described under the name "phagocytosis" immunity and recovery from bacterial invasion. This theory is based on the well-known fact that certain cells of the body have the power of surrounding and ingesting foreign substances. The cells in question are chiefly polynuclear leukocytes, but to some extent other leukocytes and endothelial and other cells are also concerned. The polynuclear leukocytes are the cells which destroy bacteria, and Metchnikoff now calls these *microphages;* other phagocytes he calls *macrophages.* There are many examples of phagocytosis which have been observed. The phagocytes of the lungs constantly take up small bits of carbon inhaled with the air. Particles of carmine injected into the tissues will later be found within phagocytes. After a hemorrhage, phagocytic cells may be found containing red blood-corpuscles or particles of blood-

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*Greek, *φαγεῖν*, to eat; *κύτος*, a cell.

pigment. The presumption is that phagocytic cells serve to remove irritating and foreign bodies and to destroy them. Metchnikoff showed that phagocytes also absorb bits of degenerating or useless tissue. Such particles disintegrate, and they are digested and become a part of the protoplasm of the phagocytes. This process is seen when the tail of the tadpole shortens. The superfluous part is absorbed, at least in part, by phagocytic leukocytes. Metchnikoff's earlier observations were made largely on the invertebrates, whose transparent bodies may be studied while living. One illustration was furnished by a small crustacean (*Daphnia* or water-flea,) which was often infected with a fungus. Some infected individuals died, others recovered. Metchnikoff found that the cells of the fungus might be ingested and destroyed by the leukocytes of the *Daphnia*. He described the history of this disease as a contest between the parasitic cells and the phagocytes, in which either might succeed. Similarly, when anthrax bacilli were introduced into frogs, which are immune from anthrax, the bacilli were ingested by the frog's leukocytes. Metchnikoff* contends that this function of leukocytes and other phagocytic cells constitutes the principal defense of the body against bacteria.

Other investigators also have seen bacteria inclosed within the bodies of leukocytes. It has been urged by some that the bacteria are already dead when the leukocytes devour them, but Metchnikoff showed that these inclosed bacteria are still alive, for they produce disease when introduced into fresh animals; so they are apparently not injured before they are taken up. In other cases, as with the gonococcus, which is commonly found inclosed within leukocytes, it is quite evident from their appearance that the bacteria retain their full vigor after being ingested.

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*Metchnikoff. Comparative Pathology of Inflammation. Trans., Starling. 1893.

It is well known that a suppurating part contains large numbers of leukocytes, and one of the most characteristic events in the inflammatory process is the migration of leukocytes to the point of irritation. This indicates a positive chemotaxis for leukocytes on the part of substances in the inflamed area. Metchnikoff believes that the function of these leukocytes is to destroy the bacteria and to arrest their further progress. On this theory bacteria have often been likened to an invading army and the leukocytes or phagocytes to a force designed to repel their attacks.

It is certain that in some infectious diseases the number of leukocytes, chiefly of the polynuclear neutrophilic variety, in the circulating blood is increased (leukocytosis). This is the case usually in lobar pneumonia and acute suppurative infections. In some other infectious diseases there is usually no leukocytosis; for example, tuberculosis, typhoid fever and malaria. It is interesting to observe that in trichinosis, and more rarely in infection with other animal parasites, the eosinophilic leukocytes* become much more numerous in the blood than normally.

Manwaring and Ruh† studied the effects of various antiseptic and therapeutic agents upon phagocytosis, and found: That carbolic acid causes diminution of phagocytosis in proportion to the amount of carbolic acid present, causing complete cessation when present in the proportion of ½ per cent. That corrosive sublimate in the proportion of less than $\frac{1}{120}$ per cent. causes preliminary stimulation followed by gradual diminution. In larger amounts it causes deterioration from the start. Boric acid less than 1½ per cent. causes transient stimulation, followed by depression and complete cessation at 2 per cent. Quinine hydrochloride causes stimulation of phagocytosis up to $\frac{1}{120}$ per cent., which is the maximum effect. Larger amounts are depressing. Complete cessation at $\frac{1}{40}$ per cent.

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*Williams and Bentz. Trans. Association of American Physicians. XVIII. 1903. p. 152.

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Park and Biggs. *Jour. Med. Res.* Vol. XVII., No. 1.

†*Journ. Exper. Med.* Vol. IX. pp. 473–486. 1907

Experiments have been made by various investigators, consisting in the production of local leukocytosis, and studying the effects of the leukocytes thus brought together upon bacteria. The injection into the pleural or peritoneal cavity of various substances, notably nucleic acid, or aleuronat suspensions, calls forth a great accumulation of leukocytes, and these masses of leukocytes have been used for the purpose of observing the phenomena of phagocytosis both inside and outside the body.*

In operations upon the abdominal cavity the production of artificial leukocytosis in the peritoneal cavity previous to operations has been suggested and tried with apparent success on the ground that if any bacteria entered during the operation they would be destroyed by the phagocytes.†

Rubin‡ found that narcotics, alcohol, ether, chloroform, appear to directly lessen the affect of the substances which inhibit the growth and toxic action of the bacteria in the bodies of normal animals.

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*Gustav F. Ruediger. The Mechanism of Streptococcus Infection. *Journal of the American Medical Association.* No. 3, January 21, 1905. p. 198. Ludvig Hektoen and Gustav F. Ruediger. Studies in Phagocytosis. *Journal of Infectious Diseases.* Vol. II., No. 1, p. 128 January, 1905.

†Von Mikulicz. Versuche über Resistenzvermehrung bei Magen und Darmperforationen. *Archiv für klinische Chirurgie.* Bd. LXXIII., Heft 2, p. 347. 1904.

‡*Journ. Infectious Diseases.* Vol. I., 1904. pp. 425–444.

On the other hand, Wright and Douglas,* whose results have been corroborated by others, found that certain substances prepare the bacteria for the phagocytes. These substances are developed in the blood under certain conditions. Thus, injections of dead cultures of the S. pyogenes aureus into the blood produces a substance which prepares the live staphylococcus as food for the phagocytes. Substances acting in this way they call "opsonins" (*opsono*, I prepare victuals for).

These authors find that phagocytosis for certain organisms depends upon the presence of opsonins in the blood. Thus, B. typhosus, S. choleræ Asiaticæ, B. coli communis, B. dysenteriæ, S. pyogenes aureus, B. pestis, M. melitensis, D. pneumoniæ, are all taken up by phagocytes after being prepared by the opsonins. B. diphtheriæ and B. xerosis are not acted upon by opsonins.

Wright speaks of the number of bacteria which may be counted inside of the leukocytes on an average as the "opsonic index," and he uses so-called vaccines derived from cultures with the purpose of increasing this index. There is wide diversity of opinion in regard to the value of the whole opsonic theory, both for diagnostic and for therapeutic purposes, and the stronger authority seems opposed to attributing very great value to the use of opsonins for therapeutic purposes.

Hektoen† finds that the opsonic function of normal and immune serum is due to a distinct body, different from lysins and agglutinins.

Buxton‡ finds support for Wright's view that the opsonins are a very important factor in immunity. The influence of the opsonins on the bacteria is to make them more acceptable to

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*A. E. Wright and Stewart R. Douglas. An Experimental Investigation of the *Rôle* of the Blood Fluids in Connection with Phagocytosis. Proceedings of the Royal Society. Vol. LXXII., No. 483, p. 357. October 31, 1903. *Ibid.* Vol. LXXIII., No. 490, p. 128. February and March, 1904.

†Hektoen. *Journ. Infectious Diseases.* Vol. III, 1906. pp. 434–440.

‡*Journ. Med. Research.* V. XVI., No. 2, 1907. p. 264.

the makrophages; with this influence, however, the power of the opsonins seems to terminate, for the makrophages of the immune animal do not seem to destroy the bacilli more quickly than the normal one. It may be that the opsonins indirectly protect the immune animal, since where phagocytosis is active the endotoxins are liberated inside the phagocytes, and not in the circulating plasma.

Neufeld and Rimpau* found that antistreptococcus serum acts as an opsonin.

Just the contrary effect to opsonins, on the other hand, is produced by certain other bodies; for Bail† has found that when tubercle bacilli undergo bacteriolysis certain substances are liberated which check phagocytosis. These he regards as endotoxins, and gives to them the name *aggressins.*

There can be no doubt but that phagocytosis plays an important part in combating bacterial infection. And what follows in regard to the germicidal and antitoxic action of the fluid portion of the blood containing no phagocytes does not alter the fact that phagocytes, when these are present, do destroy the bacteria. Moreover, Metchnikoff maintains that the germicidal property of the blood-serum free from cells is due to substances liberated from the phagocytes by phagolysis, or breaking up of phagocytes. In other words, he holds that infection is combated by the phagocytes or by substances derived from the phagocytes.

But be this as it may, it is well established that the serum of the blood deprived of leukocytes also has the property of destroying bacteria in many cases. The effect produced upon the bacteriolytic property of the blood-serum by injecting the animal from which the serum is obtained with bacterial cultures has already been stated (page 210).

***Deutsche medicinische Wochenschrift.* 1904. Bd. XXX., p. 1458.

†Oskar Bail. *Wiener klinische Wochenschrift.* Bd. XVIII., 1905. Review in *Bulletin de l'Institut Pasteur.* III., p. 348. 1905.

It has already been explained that the neutralization of bacterial poisons or toxins takes place by the production of antitoxins (page 209), and that the gradual injection of toxins is followed by a greatly increased production of antitoxin. Bacteriolysins, then, may be produced—or at least their production may be stimulated—by injections of bacteria, while antitoxins are produced by injections of toxins; and Ehrlich advanced his now celebrated side-chain theory to explain these phenomena as well as the formation of agglutinins, lysins and precipitins.

Ehrlich's Side-chain Theory of Immunity.*—Ehrlich was the first to offer an explanation from a chemical point of view of the action of toxins on cellular protoplasm and the formation of antitoxins. He assumes, to begin with, that the molecules of the protoplasm are to be regarded as being endowed with chemical groups, present in the form of lateral appendages to the molecule, called side-chains. They can be illustrated by the analogies presented by the graphically written formulæ of some complex molecules. It is necessary to conceive of molecules made of an immense number of atoms, and bristling with projecting side-chains. The function of the side-chains is to become attached to other organic molecules with which they have affinities. In this manner they aid in absorbing the substances essential for the nutrition of the protoplasm of cells.

The side-chains are therefore also called "*receptors*"—a more appropriate name. The numerous receptors which a molecule

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*The literature of this subject is very extensive. An exhaustive review is that by L. Aschoff. Ehrlich's Seitenkettentheorie. *Zeitschrift für allgemeine Physiologie.* 1902.

The following are also of a general character: Ehrlich's Croonian Lecture. Proceedings of the Royal Society. LXII., p. 437. 1900. Ehrlich. Schlussbetractungen. Nothnagel's System of Medicine. Vol. VIII. H. C. Ernst. Modern Theories of Bacterial Immunity. 1903. Prudden. *Medical Record.* February 14, 1903. Ritchie. *Journal of Hygiene.* Vol. II. 1902. Bergey. *American Medicine.* October 11, 1902. Immunity. Special Article. *Journal, of the American Medical Association.* No. 4 *et seq.* 1905.

has are of many kinds, with affinities for other molecules of different kinds. Each kind of receptor will then have an affinity for a molecule of a particular kind, which it may be said to "fit," as a key fits in a lock, although this expression must not be taken in a literal sense. A receptor to which tetanus toxin might become attached would not "fit" diphtheria toxin. In order that toxins may be able to combine with the receptors, their structure must be nearly like that of the food molecules which the receptors are adapted to receive.

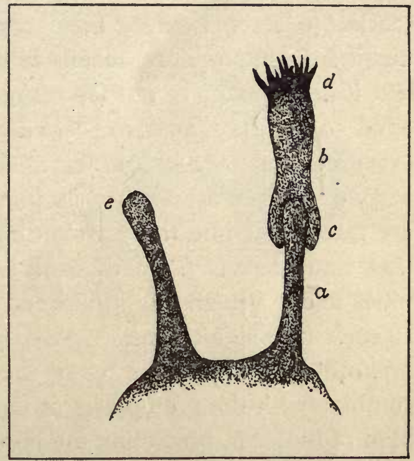

Fig. 50.—Receptors of the first order uniting with toxin.—(*Journal of the American Medical Association.* 1905. P. 955.)

a. Cell receptor. *b.* Toxin molecule. *c.* Haptophore of the toxin molecule. *d.* Toxophore of the toxin molecule. *e.* Haptophore of the cell receptor.

Secondly, soluble toxins are to be looked upon as definite chemical bodies excreted by bacteria, and containing two essential groups of atoms. One group is the *haptophore*, by means of which the toxin may be linked with the receptors of the molecules of the cell. The other group is the *toxophore*, which is capable of destroying the protoplasmic molecule, after attached to the receptor of the latter by the haptophore.

These relations have been represented schematically. In Fig. 50 a portion of a cell is shown, with receptors. A molecule of toxin, *b*, is attached by its haptophore, *c*, to the haptophore of the cell receptor, *a*. A free cell receptor is also shown with its haptophore, *e*, capable of uniting with any toxin molecule that may be present. The toxin molecule, *b*, has its toxophore group represented by the fringe-like end, *d*. If the cell receptor becomes detached from the cell, its haptophore, *e*, may unite with a toxin molecule as readily as when the receptor is still attached to the cell. Such a detached receptor constitutes a molecule of antitoxin.

As the side-chains or receptors of the protoplasm are essential to its existence, their combination with the toxin, through its haptophore, results in destruction of the molecule. But if the damage be not too serious, the protoplasm is stimulated to produce numerous similar side-chain groups—to an overproduction of these, in fact. As not all of these are necessary for the performance of its functions, the superfluous ones are thrown off into the surrounding serum. It is well known that many cells of the body exhibit analogous heightened activities under stimulating influences, as pointed out by Weigert. If such free side-chains or receptors combine with the haptophorous groups of the toxin, the latter is no longer able to combine with the protoplasm of the cells. Thus they act as a kind of buffer in protecting the protoplasm from the attacks of the toxins. These free, cast-off receptors constitute the antitoxic part of the serum as stated.

Numerous experiments have been made which illustrate the probable chemical nature of antitoxin action. A fatal dose of diphtheria or tetanus toxin may be neutralized outside of the body by mixing it with its appropriate antitoxin. Injection of the mixture shows it to be innocuous to animals.

The manner in which toxins combine with protoplasm has been shown in the case of tetanus toxin. The filtrate from cultures of tetanus bacilli will kill guinea-pigs, presumably by damage to the central nervous system. The same filtrate rubbed up with brain or spinal cord has been found to have lost its toxic properties. It may be assumed that the poison has combined with the protoplasm of the cells.

But the side-chain theory offers an explanation not only of the mechanism of the union of toxin and antitoxin, but also explains the phenomena of agglutination, precipitation and cytolysis. In the union of antitoxin and toxin, as stated above, the process is a simple combining of the toxin with the receptor, and there the process ends. Receptors of this kind are called receptors of the first order (Fig. 50). But after the union of the agglutinins and of the precipitins with their receptors further change takes place. In the one case, clumping; in the other, precipitation; and these changes are brought about by a kind of fermentative action. So, in addition to the haptophore group, the receptor must possess a ferment-producing group. It seizes on the red cells or on the bacteria, as the case may be, with the haptophore group, and produces certain changes with its ferment-producing group. The latter is called the zymophore group. Receptors of this kind are called receptors of the second order (Fig. 51).

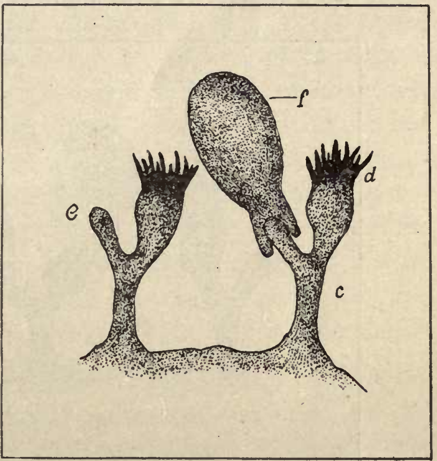

FIG. 51.—Receptors of the second order and of some substance uniting with one of them.—(*Journal of the American Medical Association*. 1905. P. 1113.)

c. Cell receptor of the second order. *d*. Toxophore or zymophore group of the receptor. *e*. Haptophore of the receptor. *f*. Food substance or product of bacterial disintegration uniting with the haptophore of the cell receptor.

With the lysins there is also a change, which takes place after the receptor unites with the bacteria or other cells; so there must be here also a zymophore or zymotoxic group, as it is called. This zymotoxic group, however, is not an integral part of the receptor, but is easily broken off from it, in the manner described below.

As is explained later, the power of the lysins becomes suspended, but not lost, on being heated to 55° or 56° C. In this condition they are said to be inactivated. They become active again when certain fresh serum is added—not necessarily fresh lysin, but fresh normal serum. This will all be discussed and explained later. For the present purpose it is sufficient to bear in mind that lysin becomes inactivated and may be reactivated. So the lysins act differently from agglutinins and precipitins. They must have peculiar receptors which unite, on the other hand, with the cells which they disintegrate and, on the other, with a ferment-producing substance easily destroyed by heat. These receptors must possess two haptophore

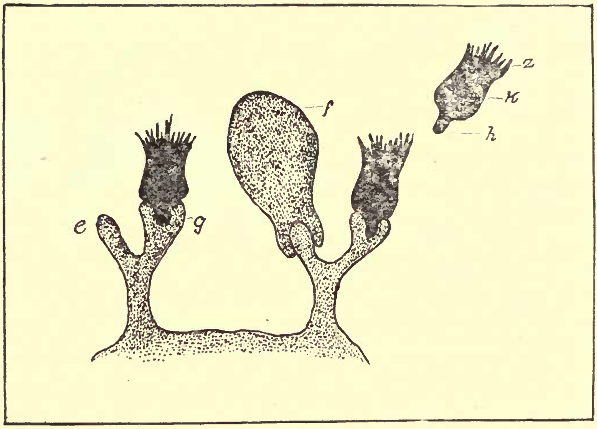

FIG 52.—Receptor of the third order, and of some substance uniting with one of them.—(*Journal of the American Medical Association.* 1905. P. 1369.)

c. Cell receptor of the third order—an amboceptor. *e.* One of the haptophores of the amboceptor, with which some food substance or product of bacterial disintegration (*f*) may unite. *g.* The other haptophore of the amboceptor with which complement may unite. *k.* Complement. *h.* The haptophore. *z.* The zymotoxic group of complements.

groups; in other words, a haptophore for cells and a haptophore capable of uniting with a body containing a ferment-producing group. Receptors for lysins are therefore called amboceptors, or receptors with two haptophores (Fig. 52). They are also called receptors of the third order. The substance which reactivates the lysin, the fresh serum, is called complement, and it must be composed of a haptophore in order to attach itself to the amboceptor, and a zymotoxic group in order to produce lysis. On heating fresh normal serum to 55° or 56° C. the complement which it contains is not destroyed, but its zymotoxic group alone is destroyed; the haptophore group, on the other hand, resists heat. So if heated complement be added to inactivated lysin, it unites with the freed haptophore. A lysin inactivated by heat with fresh serum added disintegrates homologous cells; but a lysin inactivated by heat when heated fresh serum is added will not only not produce lysis of homologous cells, but will not do so even when unheated fresh serum is subsequently added.

The behavior of mixtures of toxins and antitoxins is most peculiar, for they do not in all cases obey the simple rule of relative proportion. It is true that if a certain amount of antitoxin neutralizes a certain amount of toxin, then any multiple of this amount of antitoxin will neutralize the same multiple of toxin if the two are mixed all at once. So far the rule is simple. But if 100 doses of toxin—*i. e.*, enough to kill 100 guinea-pigs—is exactly neutralized, and then the amount of free toxin necessary to kill a guinea-pig is added, it will not kill a guinea-pig as would be expected. Many doses have to be added, sometimes as much as thirty or forty doses or more, before the mixture again becomes lethal.

Another remarkable property is that toxin that has stood for a long time loses greatly in poisonous properties, but not in its power of combining with antitoxin. Furthermore, this old toxin will produce antitoxin if injected into horses or other susceptible animals.

In order to explain these extraordinary reactions several theories have been advanced, and in this connection certain peculiar reactions obtained with lysins, agglutinins and precipitins, which have helped to give an insight into the processes involved, have also been explained on similar theoretical grounds. These theories will now be discussed.

Ehrlich regards the beef-broth from a diphtheria or tetanus culture as a solution of several different but related bodies, and he makes so-called "spectra" to explain this idea. He thinks that primarily the substances are toxin and toxon (Fig. 53), each having affinity for antitoxin; but the affinity of toxon for antitoxin is weaker than the affinity of toxin for the antitoxin. And, furthermore, toxon—no matter in what dose—does not kill guinea-pigs quickly if at all, but causes a paralysis of the animal some weeks after inoculation, while toxin, on the other hand, in just the proper amount kills a guinea-pig weighing 250 grams in two days. This is the standard minimum fatal dose, or 1 d. l. (dosis letalis).

Now, if enough antitoxin is added to the poisonous beef-broth it will neutralize both the toxin and the toxon, but if not enough is added for this, the toxin is first neutralized and the toxon still produces paralysis of the guinea-pig.

But on standing, both toxin and toxon quickly become changed; a part of the toxin is converted into a body called toxoid and a part of the toxon into toxonoid, and, while retaining their affinity for antitoxin, these are both weakened in pathogenic power as compared with the original toxin; toxoid, in fact, is devoid of toxic properties. Toxoid, then, is toxin deprived of its toxophore, but it retains the haptophore group. Still further, the resulting toxoid and the toxon have each three different degrees of affinity for antitoxin.

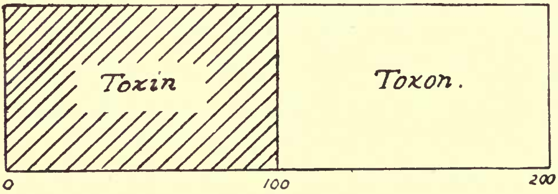

FIG. 53.—"Spectrum" of theoretically fresh, crude toxin. Such a combination probably does not occur.

A part of the toxoid has less affinity than the toxin; a part equal; a part more. These are designated epitoxoids, syntoxoids and protoxoids, respectively (Figs. 54 and 55), and toxons are in like manner designated epitoxons, syntoxons and protoxons. But, since all toxons have less affinity for antitoxin than toxin has, it follows that epitoxoid and toxon are the same. All crude toxin, then, is composed of a mixture of toxin, toxoid and toxon, for toxoids begin to form immediately, so that toxin-toxon without toxoid is not known.

When enough antitoxin is added to 100 doses (100 d. l.'s) of crude toxin to just neutralize it, all the toxin and all the toxon are united to antitoxin. But if fresh toxin is added, some of the toxoid and toxon is liberated, and the added toxin becomes attached to the antitoxin in its place; and so with each additional amount of toxin added more toxon and toxoid is liberated till the point is reached where all the toxon and toxoid is free, and the additional toxin finds all the haptophores of antitoxin occupied by the toxin previously added. In this case any additional toxin remains uncombined, and, if such a mixture is injected into a guinea-pig, the animal is killed.

Bordet's* explanation differs from Ehrlich's. Bordet does not admit the existence of toxons, and regards the paralysis attributed by Ehrlich to the action of this hypothetical substance as due to weakened toxin. He explains the peculiar behavior of a neutralized mixture of crude toxin with antitoxin, stated above, by assuming that antitoxin is capable of taking up and neutralizing varying amounts of toxin.

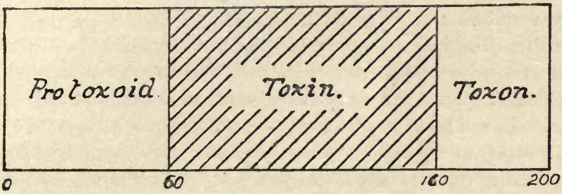

FIG. 54.—"Spectrum" of very fresh crude toxin.

He compares the effect of mixing toxin and antitoxin to that of mixing starch and iodine: the more iodine added to the starch, the bluer the color. Let A represent, then, a certain amount of antitoxin; let A be capable of combining 1, 2, 3, 4, 5, different amounts of toxin; call these amounts of toxin T_1, T_2, T_3, T_4, T_5; and assume that a combination in which all the A's are combined with T's in

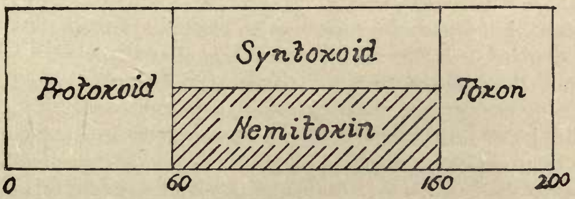

FIG. 55.—"Spectrum" of crude toxin as it is supposed practically always to occur.

the proportion of AT_1, is neutral, that it has no poisonous properties; that a combination represented by AT_2 also has no toxic properties, but that AT_3, would begin to show toxic properties, and that AT_4 is distinctly toxic, and that AT_5 is very toxic. Starting with toxin, then, if just enough antitoxin is added to neutralize its poisonous properties, AT_1 is first formed, which is not toxic; now add more toxin, and none of this remains free, but, on the contrary, AT_2 is formed, which is not toxic; on adding still more, when AT_4 or AT_5 is reached

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*Bordet. Toxines et Antitoxines. *Annales de l'Institut Pasteur.* 1903. p. 161 *et seq.*

the mixture is fatal for guinea-pigs. The paralysis which Ehrlich attributes to toxon would be represented, say, by AT_3. In between the the combinations represented by AT_1 to AT_5 are all imaginable combinations, a sliding scale of no definite units.

In other words, while Ehrlich holds that toxin and antitoxin unite in one definite proportion. Bordet holds that they may unite in any proportions, like two different colors of paint mixed together producing any intermediate color with more or less tint of one or other of the original colors.

The evidence adduced by Bordet for this conception is very abundant and fully repays study.

Still another theory offered to explain the peculiar behavior of the antitoxin-toxin mixture is advanced by Arrhenius and Madsen, also supported by experimental evidence. They also deny the existence of toxon, and look upon a mixture of antitoxin and toxin as analogous to an amphoteric mixture of a dilute acid and alkali, or of an acid and alcohol. In such combinations there are compounds formed of the two substances, but some of each of the two constituents remains free. An ester is not only a compound formed by an acid and an alcohol, but it has free alcohol and free acid. Moreover, the ester is constantly changing, some of the alcohol and some of the acid separating and new ester constantly forming again. When first mixed, more ester is formed, and less alcohol and acid are liberated, till a point of dynamic equilibrium is reached, when just as much ester is formed as there are alcohol and acid liberated. Just so in adding toxin to antitoxin: at first more of the two combine than is set free, but after a time a condition of dynamic equilibrium is established, and any additional toxin remains free.

Briefly stated, these are the three theories which are now advanced by competent authorities, and, if these outlines are kept clearly in mind, it will not be difficult to understand the subject as presented in the many medical journals and the many monographs which have appeared on the subject.*

The theories of Ehrlich and Bordet in regard to the composition of lysins may also be appropriately discussed in this connection, as it is from the studies of these bodies that many of the ideas in regard to immunity have been developed.

A lysin contains two substances, a thermolabile and a thermostabile substance —*i. e.*, one readily destroyed by heating at 55° C. for a half-hour, the other resisting much higher temperatures. The thermostabile substance is now called by Ehrlich the *immune body*, the thermolabile the *complement*, though Ehrlich has used in the past various other names for these hypothetical bodies. Bordet uses the name *substance sensibilisatrice* for the thermostabile, Ehrlich's immune body, and *alexin* for the thermolabile or Ehrlich's complement. Both are agreed that

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*A summary will be found in a monograph by Michaelis. Die Bindungsgesetze von Toxin und Antitoxin. Berlin. 1905. Also a special article entitled. Immunity. *Journal of the American Medical Association.* Nos. 4 *et seq.* 1905.

there are two bodies concerned: both are agreed as to the property of the one to be readily destroyed by heat, of the other to resist heat.

In English writing it is more common to use the German than the French terms, so these will be employed in the present case, though a great deal of what is known about lysins has been contributed by Bordet and the French school generally. The word alexin was first used by Buchner, but is used now mostly in French writings.

It should be recalled that a lysin is the substance formed in the blood-serum of an animal when the latter is injected with bacteria or with foreign red blood-cells. A rabbit injected with typhoid bacilli develops lysin for typhoid bacilli; when injected with red blood-cells of a guinea-pig, develops a lysin for guinea-pig red cells. Lysins are not only produced artificially by such injections but they may also be present in blood-serum normally.

If the lysin is heated to 55° C. for thirty minutes, it loses its complement (or *alexin*) and the immune body (*substance sensibilisatrice*) only remains; so that red cells which are disintegrated by the unheated lysin remain intact in the heated lysin. But the heated lysin becomes active again if either fresh unheated rabbit's or guinea-pig's serum is added to it. The heated lysin is spoken of as inactivated-the heated lysin with fresh serum, reactivated. The fresh serum which is added contains the complement (*alexin*); the heated lysin contains only the immune body (*substance sensibilisatrice*).

The immune body is specific, but the complement is not; at least the blood of some animals contains complements for several different immune bodies. Thus fresh horse serum added to various inactivated lysins reactivates the latter. But chicken blood-serum does not contain complement for chicken corpuscles. For if chicken hemolysin, produced by injecting a rabbit with chicken red cells, is heated to 55° C. for thirty minutes (inactivated), it will not disintegrate chicken red cells if fresh chicken serum be added; but if fresh rabbit serum is added, it will hemolize chicken red cells as it did before heating.

The immune body becomes fixed to the red cells, as can be shown by adding red cells to inactivated lysin and then washing these with salt solution. If after adding the red cells to the inactivated lysin the mixture is centrifugalized and the precipitated red cells washed with salt solution so as to remove all of the free immune body, the precipitated, washed red cells disintegrate when fresh complement—*i. e.*, fresh serum—is added.

From these and other considerations Bordet regards lysin as composed of a specific antibody, *sensibilisatrice* or immune body of Ehrlich, on the one hand and of a cytolytic, bacteriolytic, hemolytic alexin proper or complement of Ehrlich, on the other. The immune body is specific, but it does not cause destruction of cells by itself: it does so only in conjunction with complement or alexin. Alexin is not strictly specific, and it has some cytolytic power, as seen in normal blood independently of *substance sensibilisatrice.* But its power is greatly enhanced if the cells acted upon are first sensitized by *sensibilisatrice.*

The reactions found by Bordet may be briefly summarized as follows:

Bacteria or other cells which are united to the same immune body or *sensibilisatrice* become disintegrated upon the addition of diverse complements.

In any one cytolytic serum the complement for bacteria and for red cells is one and the same. The fixation of the immune body takes place by the stroma of the red cells, for red cells washed of all their contents so that only the empty capsules are left fix the immune body just as well as unwashed red cells.

Antilysin combines with both immune body and complement.

Since antilysin neutralizes the complement, it is both antihemolytic and anti bacteriolytic, because the complement for both of these is the same.

Pfeiffer was the first to describe the bacteriolytic action of animal fluids on bacteria, and the reaction is called Pfeiffer's phenomenon. Pfeiffer's view of the nature of lysin differs from those of Ehrlich and of Bordet. He holds that lysin is not composed of two bodies, but that it is one body which is readily changed into an active and passive condition. This view does not seem to have found as much favor as that of Ehrlich and that of Bordet.

But among those who have accepted more or less completely the Ehrlich conception of the nature of the bodies concerned in bacteriolysis, there is difference of opinion as to the mode of action of the complement. Ehrlich himself says in regard to the matter "that one will not go amiss if he assumes with Pfeiffer that the process of bacteriolysis is analogous to digestion, and attributes to the addiment (complement) the character of a digestive ferment." Gruber,* on the contrary, contends that the complement does not act like a ferment, and that it is erroneous to draw any analogy between the complement and an enzyme, since the complement is entirely used up in bacteriolysis, whereas in the process of fermentation, as is well known, the ferment is not used up, but may be recovered after the action is ended and used for the fermentation of other material.

But whatever the exact mode of action may be, it is evident from what has just been said that both the Ehrlich and the Bordet schools attribute bacteriolytic action proper in normal serum to a substance easily changed by comparatively low temperatures, and called, respectively, complement and alexin

*Gruber. Kolle and Wassermann's Handbuch der pathogenen Mikroörganismen.

by the two schools. To the other body concerned in bacteriolysis—the amboceptor of Ehrlich and the substance sensibilisatrice of Bordet—is assigned by the former the rôle of a binding link between the complement and the bacterium, while by the latter is assigned to it the property of a sensitizer or of a mordant as in dyeing. In the one case the bacterial cell is regarded as not at all injured or otherwise changed by the union with the amboceptor; in the other case it is the opinion of those holding this view that the cell is acted upon and changed by the sensibilisatrice in such a way that the alexin can penetrate it.

Bordet* summarizes the difference between his theory and that of Ehrlich as follows:

> According to Ehrlich and Morgenroth the specific antibody (sensibilisatrice) plays the rôle of an actual intermediary (zwischenkörper, amboceptor), a link of union attaching itself on the one hand to the cell, on the other to the alexin. In other words, the absorption which the alexin undergoes in the presence of the sensitized cell is not due to an affinity manifested by the cell itself to this substance. The absorption of the alexin is only indirect; the cell joins itself to the intermediary substance, which is itself united chemically by its other pole to the alexin.
>
> Our idea of the phenomenon, which we feel we are justified in holding, is altogether different from this. To us it seems that the sensibilisatrice which unites with the cell modifies this in a way which permits it to absorb the alexin directly. The action of the sensibilisatrice upon the cells is comparable to that of certain fixative agents or mordants which confer upon certain substances (or to the cell of these substances, as is the case in histological technic) the power of absorbing colors which they refuse to absorb before treatment. * * * It is to be clearly understood, however, that when we speak of mordants in this connection we do not intend to apply in all details the phenomena of dyeing to the matter at present under consideration; we merely mean to draw a comparison which will serve to make our idea clearer. The hypothesis which we wish to bring out in relief is that in the presence of hemolytic serum, the cell becomes capable of absorbing directly the alexin by means of its own proper elective affinity, and that this power is due to a change caused by the sensibilisatrice. In other words, we do not believe that one is forced to admit, with Ehrlich and Morgenroth, that the sensibilisatrice itself combines with the alexin, and that this union is indispensable for the latter substance to attack the cell.

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*Bordet, Jules. Sur le mode d'action de sérums cytolytiques et sur l'unité de l'alexine dans un même sérum. Ann. de l'inst. Past., T. 15, no. 5, 303–318. Paris, May 25, 1901.

Bordet furthermore states in the same connection that he agrees with Buchner in regarding the alexin for blood-cells and for bacteria as identical—that one and the same alexin may attack the most diverse cells; whereas Neisser and others of the Ehrlich school hold that alexins or complements are different in one and the same serum.

While it is evident from the above that the terms amboceptor and sensibilisatrice are used to designate the same substance, it is scarcely correct to use them interchangeably, since they connote somewhat different attributes in the body to which they refer. The same is true of the terms complement and alexin, though to a less degree.

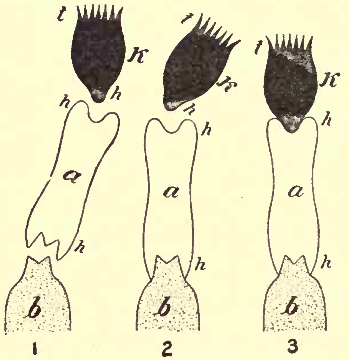

Fig. 56.—Illustration of the mechanism of bacteriolysis according to the Ehrlich hypothesis.

The following diagrams, obtained from various sources and modified to suit the purpose, will serve to illustrate the process of bacteriolysis according to the view of the Ehrlich school.

Fig. 56 represents in its simplest form the mechanism of bacteriolysis according to the Ehrlich hypothesis, and serves to illustrate the process sufficiently for present purposes.

In the diagram the bacteria are represented by the parts marked *b*, the amboceptors by those marked *a*, and the complements by those marked *k*. In No. 1 the bacterium, amboceptor, and complement are represented as just on the point of uniting. No. 2 represents the bacterium and the amboceptor united and the complement on the point of uniting with the unoccupied end of the amboceptor. No. 3 represents the process of uniting of bacterium, amboceptor and complement completed; the bacterium in this case would undergo bacteriolysis.

It should be borne in mind that according to this theory bacteriolysis can take place only where the bacterium becomes united to an amboceptor which is itself united with a complement. A bacterium may become united with a free amboceptor—*i. e.*, an amboceptor which is not united with a complement—but the bacterium in such a case does not undergo bacteriolysis unless a complement subsequently becomes attached to the amboceptor. The complement is incapable of uniting directly with a bacterium; it can do this only through the intervention of the amboceptor. But when the complement becomes linked to the bacterium by means of the amboceptor, the bacterium becomes broken up into minute granules and ultimately disappears.

The bonds by which the amboceptor attaches itself to the bacterium on the one hand, and to the complement on the other are called haptophor groups or haptophors (*h*), and similarly this name is given to the bonds of union of the bacteria and of the complement. The amboceptor thus has two haptophors, one by means of which it attaches itself to the bacterium, the cytophylic haptophor, and one by means of which it attaches itself to the complement, the complementophylic haptophor. The bacteria probably possess each many haptophors, all of the same kind—*i. e.*, haptophors capable of uniting with amboceptors of the same kind—but for the sake of simplicity the bacterium is represented in the diagram as having only one haptophor. The complement has one haptophor group and one so-called toxophor group (*t*), and it is by means of the latter group that the complement acts upon the bacterium. The complement may be deprived of this toxophor group, and although it is still capable of uniting with the amboceptor in such a case, it can no longer cause bacteriolysis. This loss of the toxophor group is caused by heating, and is also occurs spontaneously in the serum on standing. Bacteria subjected to the action of heated serum do not undergo bacteriolysis, but become fixed to the amboceptors, and the amboceptors become united to the haptophor group of the complement which are left unaffected by the heating. It will thus be readily understood why bacteria treated with heated immune serum are subsequently protected from bacteriolysis even when unheated immune serum or when unheated complement is added to them. The complementophylic haptophor of the amboceptor is in such a case already occupied by the haptophors of the heated complement, which has in this way become deprived of its toxophor group.

The amboceptors found in ordinary normal serum are either all alike—and in this case they must possess affinity for a great many different kinds of bacteria—or they must differ from one another; and in this case there must evidently be a great many specific amboceptors, some fitted for the bacteriolysis of one species of bacterium, some for others. This matter seems not yet to have been settled. But it is certain that the injection of an animal with certain bacteria or their products causes the formation of a large number of specific amboceptors; that is to say, of amboceptors having affinity only for the kind of bacteria with which the animal is injected. Such injections seem not to increase the amount of complement.

Complement is found normally in the serum, that of some animals possessing more than that of others. The horse appears to have a large amount of complement in the serum. It is not yet settled whether the complement is specific—that is, whether the complement for one kind of immune serum can unite with the amboceptors of this serum only and not with the immune serum of a different sort—or whether complements are general; though they seem for the most part not to be specific. The complement in the serum of horse's blood seems capable of reactivating heated immune serum of various kinds. Still in some cases it would appear as if they were specific.

With the explanation given above of the nature of amboceptors and complements, the phenomena which take place in immune serum become more or less satisfactorily explicable. By means of the characteristics ascribed to these bodies it is possible to account for the peculiar behavior of immune serum when it is diluted. This peculiar behavior consists in the fact that such serum is frequently more potent when diluted than when it is undiluted.

Neisser and Wechsberg were the first to offer a theoretical explanation of this phenomenon, and they very appropriately call their explanation the theory of the diversion of complement.

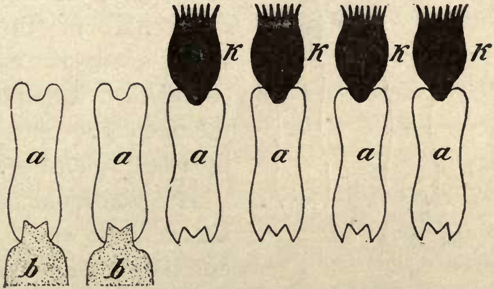

FIG. 57.—Diversion of complement in undiluted immune serum.

As the name implies, they attribute the lack of bacteriolysis in the undiluted immune serum to the turning aside of the complement from the bacteria, or rather from the amboceptors which are attached to the bacteria. They hold that this diversion is brought about by the free amboceptors themselves. In other words, where there are more amboceptors than there are complements present in a serum, a part of these attach themselves to the bacteria and a part to the complements.

The accompanying diagram (Fig. 57), taken from Neisser and Wechsberg, and modified to suit the present description, shows two amboceptors, *a*, attached to bacteria, *b*, and four amboceptors attached to complements, *k*. Bacteriolysis is not possible in such a condition, because the complements have been diverted from the amboceptors which are attached to the bacteria. Bacteriolysis can take place only when the complement becomes attached to the bacterium through the medium of the amboceptor.

Fig. 58 is meant to show the same serum diluted with an equal amount of salt solution. In this case, with the same number of bacteria added, it is evident that one-half of them would be killed, as is indicated by the combination between bacteria, amboceptor, and complement in Fig. 56. The other half of the bacteria would evidently escape, the complement being diverted by the free amboceptor, as shown by Figs. 57 and 58.

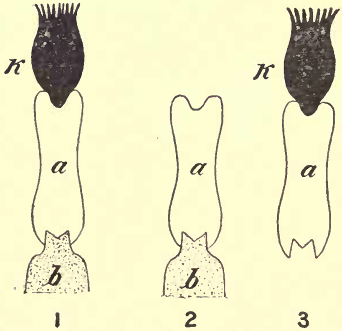

FIG. 58.—Partial bacteriolysis and partial diversion of complement in diluted immune serum.

The conditions under which bacteriolysis would take place and those under which no bacteriolysis would take place in any given serum, may be summarized as follows:

1. Complete bacteriolysis could take place only where there were no free amboceptors and where there were at the same time a number of amboceptor-complements equal to or greater than the number of bacteria introduced.

On dilution in a serum of this kind there would be a loss of bacteriolytic power in proportion to the degree of dilution if the amboceptor-complements were exactly equal in number to the bacteria introduced. If there were more amboceptor-complements originally in the undiluted serum than the bacteria introduced, then on dilution there would be relatively more bacteria destroyed. If the excess of amboceptor-complements is large enough, there could of course be enough present in the diluted serum to kill as many bacteria as were killed by the serum before dilution.

2. Partial bacteriolysis would follow when there were fewer amboceptors present in the serum than the bacteria introduced and when at the same time there were any amboceptor-complements present.

The extent of bacteriolysis upon dilution would depend upon the number of amboceptor-complements present originally.

3. No bacteriolysis could take place if the free amboceptors were equal in number to the bacteria introduced, or if they were in excess of this number, either in the undiluted or the diluted serum.

In the above discussion it is assumed that the two bodies concerned—the amboceptor and the complement of Ehrlich, the sensibilisatrice and the alexin of Bordet—are capable of uniting and do actually unite independently of the presence of bacteria or of other cells. But Bordet* nevertheless published a series of investigations tending to show that the experiments of Ehrlich and Sachs, which constitute the chief evidence in favor of this view, are capable of quite a different interpretation from this, and that this interpretation is, in fact, not justifiable from the results of the experiments which consisted in the demonstration of the fact, not denied by Bordet, that ox serum will produce cytolysis only when the serum has in it amboceptors and complements simultaneously. It is not possible, as in some other cases, to produce cytolysis by sensitizing cells with ox amboceptors—that is, with heated ox serum—and, after washing these sensitized cells, adding complement—that is, fresh serum. Cytolysis with ox serum takes place only when

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*Bordet, Jules, and Gay, Frederick P. Sur les relations des sensibilisatrices avec l'alexine. *Ann. de l'Inst. Past.*, t. 20, No. 6, p. 267–498. Paris, June 25, 1906.

the heated ox serum and some unheated fresh serum (horse serum was the kind used in the experiments) are employed at the same time. This is interpreted by Ehrlich and Sachs as showing that while the free amboceptors present in the ox serum will not unite with the cells they will and do so unite when they are previously attached to complements. But Bordet's results appear to show quite plainly that in this case the horse serum which was used as complement produces cytolysis quite independently of the ox serum, and that while cytolysis takes place more promptly when heated ox serum—ox amboceptors—are added, the ox serum is not necessary. Bordet therefore regards the experiments as showing that the heated ox serum acted merely as an accelerator of cytolysis.

Bordet summarizes his conclusions as follows:

> We see but one rational explanation of the peculiar action of ox serum—that there exists in the serum a peculiar substance capable of resisting heat of 56° C. and which remains unaltered for many months in this heated serum. The substance is probably of an albuminous or colloid character, and does not adhere to the normal corpuscles, but is precipitated upon the corpuscles which are previously charged with sensibilisatrice and alexin. We believe that it is a veritable process of glueing or absorption depending upon molecular adhesion * * * In conformity with the statements of Ehrlich and Sachs, experiments show that the corpuscles of guinea-pigs become hemolyzed in a mixture of fresh serum and of ox serum, the latter having been heated to 56° C., while they resist hemolysis if they are first subjected to the action of the heated ox serum and have the horse serum added subsequently. But the interpretation offered by Ehrlich and Sachs, according to which the sensibilisatrice furnished by the ox serum does not unite with the corpuscles unless it (the sensibilisatrice) is previously connected with alexin derived from the horse serum it not correct. In the first place, the sensibilastrice, which plays a preponderating and most essential rôle is not contained in the ox serum at all, but is furnished by the horse serum. Consequently these sensibilisatrices behave like all of their congeners, in the sense that they do not require the presence of the alexin before they are capable of uniting with the corpuscles.
>
> Finally, this interpretation leaves completely in the dark the very special peculiarities of the cases of hemolysis in question.
>
> The peculiarity of ox serum consists in the presence of a certain element which resists 56° C., and also resists standing, and is of the nature of a colloid, doubtless albuminoid, and which, furthermore, is absorbed by corpuscles which have become

> charged with sensibilisatrice and alexin but which remains free in the presence of normal corpuscles or of corpuscles merely sensitized—*i. e.*, corpuscles treated with heated serum alone. The absorption of this colloid by corpuscles which have been treated with both sensibilisatrice and alexin has the effect of enegetically agglutinating them and of rendering them more susceptible to hemolysis except under certain circumstances. * * * The absorption of the sensitized and alexinized corpuscles is very likely due to molecular adhesion, the preliminary treatment having modified the corpuscles in so far as their adhesive properties are concerned. Under these conditions the absorption may take place independently of the species of animal from which the corpuscles are obtained. it may even take place with the corpuscles of the same animal which furnishes the colloid, as in the case of ox serum.

Now, if the contention of Bordet as set forth above is correct, and if the two bodies concerned in cytolysis do not unite, then of course the theory of complement diversion must fall, since it requires as the first condition the union of amboceptors with complements. Nevertheless, there appears to be as yet no other explanation of the phenomena observed on diluting immune serum.

While it is true that immune blood-serum differs from normal blood-serum in the matter of its behavior on dilution, this difference is one of degree rather than of kind. For normal blood-serum kills relatively more bacteria when it is diluted than when undiluted.* Normal serum which will kill a given number of bacteria when undiluted will kill a great many more than $\frac{1}{10}$ that number when diluted 1:10; it will kill a great many more than $\frac{1}{100}$ of the number killed by the undiluted serum when diluted in the proportion of 1:1000. In some cases the normal serum will not only kill relatively more bacteria than the undiluted, but actually more.

*Bolton. The Bacteriolytic Power of the Blood-serum of Hogs. U. S. Department of Agriculture. *Bu. An. In. Bulletin* No. 95.

CHAPTER VII.

DISINFECTION, STERILIZATION AND ANTISEPSIS.

THE means employed for the destruction of the bacteria and for the prevention of their growth are of interest in several directions: in the prevention of the spread of infectious diseases in the avoidance of the infection of wounds, both accidental wounds and those produced in operative proceedures; and in the preservation of food-stuffs and other perishable material.

Disinfection is the term employed to signify the destruction of the infectious agent in the rooms, stables, barns, cars or other ocnfined spaces previously occupied by persons or animals suffering from an infectious disease, also the furniture in the rooms and the clothing worn by such persons. It is also used for the process employed destroying the infectious agent in feces, urine, sputa and other excreta from persons suffering from infectious disease. The term always implies the presence of an infectious agent which is to be got rid of, it is better to avoid using the term for processes employed in masking or destroying disagreeable odors arising from improperly flushed water-closets or decaying offal.

Sterilization is used to denote the destruction of bacteria in general. It does not necessarily imply the destruction of pathogenic bacteria. Thus in the preparation of culture media, the vessels and the media themselves are sterilized by means of heat, although there may or may not be pathogenic bacteria in them to start with.

Antisepsis is the term applied to the process of preventing the growth of bacteria in wounds or in articles of food or elsewhere, in other words, to the prevention of putrefaction, fermentation and wound infection. Although in practice antisepsis usually involves the destruction of bacteria as well the prevention of their growth, it does not necessarily involve sterilization. Cold prevents the growth of bacteria, and therefore acts as an antiseptic, and yet it does not act as a germicide, as will be shown later. Chloroform added to urine acts as an antiseptic, but it does not kill the bacteria, for after the chloroform is evaporated off the bacteria grow out in the urine.

The destruction of bacteria and the prevention of their growth is accomplished by both chemical and physical agents. The chemical agents are some of them applicable in aqueous solution, and some of them as gases. All agents which kill bacteria are called germicides, but their action depends upon the intensity and the length of time they are allowed to act. If they are used in dilute solutions or if they are allowed to act for a short time they may rapidly inhibit the growth or produce some change in the bacteria affecting the physiological activity of the organisms. Various permanent modifications have been produced in certain bacteria by the action of germicides applied in such a way as not to destroy the organisms outright. Thus the anthrax bacillus has been deprived of its power to form spores, it has also been made to assume graded virulence so that it would kill mice but not sheep. These modifications are produced by agencies which would destroy the bacterium of anthrax if allowed to act with sufficient intensity.

Germicides are all much more active when dissolved in water than when dissolved in any other solvent, though it is true that Epstein found that bichloride of mercury, carbolic acid, lysol and thymol were more powerful when dissolved in 50 per cent. alcohol than when dissolved in the same proportion in water, though authorities differ on this point. Solutions in oils are inert. The reason for this is that the bacterial cell is penetrated only by water, not by oil. The addition of alcohol to phenol or to formaldehyde lessens the germicidal property of these germicides. The addition of even 5 per cent. of alcohol shows this effect. On the other hand, the addition of alcohol to aqueous solutions of corrosive sublimate or silver nitrate increases the germicidal power of these germicides. The addition of glycerin to solutions of germicides acts irregularly, increasing the power in some of them, decreasing the power in others.

Researches in physical chemistry have shown that the disinfecting power of metalic salts is in proportion to their electrolytic dissociation, the more strongly dissociated a salt is by electrolysis, the stronger its disinfecting power.

Scheurlen and Spiro have shown this with iron salts. From their results it appears that only those salts of iron act as germicides in which the iron is present as kation. On the other hand, all disinfectants do not act in this way. Phenol and its derivatives do not act through the ions. In this case it is precisely those molecules which are not dissociated which act as germicides. Sodium phenolate is much more strongly dissociated than phenol itself, and yet phenol is much more strongly germicidal than the salt. But with the metalic salts anything which interferes with their dissociation weakens their disinfecting power. The addition of sodium chloride interferes with the dissociation of corrosive sublimate and weakens its germicidal power. This is shown more strongly in concentrated than in dilute solutions. In the dilute solutions of corrosive sublimate ordinarily employed in practice the addition of the salt does not weaken the germicidal power very markedly.

Temperature has a marked effect upon the action of germicidal agents. The higher the temperature, the more potent the germical action, but just the reverse is true in regard to the inhibitory action of germicides. The inhibitory action is weakest at the optimum temperature for the growth of the bacterium under observation. It is stronger in the cold. The explanation offered for this behavior is that the vigor of growth at the optimum temperature overcomes the inhibitory action of the germicide, whereas at lower temperatures the vigor of the organism is lowered, and therefore it is less capable of resisting the inhibitory action than at the more suitable temperature for growth.

The chemical composition of the medium in which the bacteria are tested may have a marked influence upon the action of germicides. If components of the medium enter into chemical union with the germicide there may be an inert compound formed. There may also be formed dense, flocculent precipitates which envelop the bacteria and protect them from the action of the germicide. It is therefore apparent that the potency of a germicide may appear very different when acting upon the bacteria in water or in physiological solution or on bacteria dried on glass rods or on silk threads, on the one hand, and upon the same bacteria in beef-broth or in feces or in urine, on the other. For these reasons it is not always possible to draw conclusions from the results of laboratory experiments as to the value of a germicidal agent for practical disinfecting purposes.

The action of germicides is still further complicated by the difference of resistance shown not only by different species of bacteria, but by the different strains of the same bacterium and even by different individuals in the same culture. Furthermore, some bacteria are more sensitive than others to certain chemical agents. In other words, some bacteria have an elective affinity for certain chemicals while others have greater affinity for other chemical agents. As examples of this elective affinity of certain bacteria for certain germicides, Gotschlich* cites the action of quinine in malaria, the mercury salts in syphilis, and the specific action of bactericidal sera.

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*Loc. cit.

The many conditions affecting the action of germicidal agents probably account for the greater or less discrepancy in the results obtained by various investigators in testing the value of disinfectants. Moreover, it has been shown that the failure of the bacteria to grow after exposure to a germicide cannot be regarded as a certain criterion of the death of the organism as is usually done; for enough of the germicide may be transferred in the inoculation to inhibit growth without actually killing the bacteria. Enough of the germicide may even adhere to bodies of the bacteria to inhibit growth and yet not kill the bacteria, as shown by ultimate growth or by growth after neutralizing the chemical agent. In all tests of the germicidal action of chemical agents it is necessary to exclude this source of error. The bacteria should be thoroughly washed after exposure to the germicide and if possible treated with some neutralizing agent which is not itself a germicide and which does not form a germicidal compound with the germicide which is being tested. The culture tubes or plates made to test the viability of the organisms after exposure must be observed for a much longer time than is done with ordinary cultures; for development may be delayed for many days and yet take place abundantly at last.

The manner in which disinfectants destroy bacteria differs in different cases. In some cases the injurious effect is due to interference with the nutrition, in some cases it is due to oxidation; but much more frequently it is due to coagulation of the protoplasm of the cell. Heat, metalic salts, phenol, all act in this way. On the other hand, not every agent which coagulates albumin acts as a powerful germicide. Alcohol and tanic acid are weak disinfectants, and yet they coagulate albumin very strongly. Lysins found in blood-serum act by breaking up and disintegrating the bacteria.

The methods employed for testing the germicidal value of any disinfectant are as follows:

1. To a measured quantity of a virulent bouillon-culture of the test-organism is added a given amount of the germicide. After varying lengths of time, inoculations are made from this mixture into culture-media, preferably bouillon, and note is made of the presence or absence of growth under suitable conditions of temperature and the like. The shortest exposure to the weakest solution of the substance necessary to kill the test-organism is taken as the germicidal value of that substance for the particular organism used.

2. Small pieces of sterile silk or cotton thread are soaked for some hours in a bouillon-culture of the test-organisms. The threads are then removed, partially dried and placed in a solution of known strength of the germicide and exposed for a definite length of time. The thread is removed from the solution, washed carefully in sterile water, dropped into a tube of sterile bouillon plugged with cotton, and growth or absence of growth noted. As in other methods, the greatest dilution of the germicide that will kill the test-organism in the shortest time is taken as the germicidal value of that substance for the organism used. Spores of the hay bacillus may be used when experiments are being made by large classes of students.

3. The test-organism is dried upon the end of a sterile glass rod contained in a sterile test-tube, the end of the rod projecting through a cotton plug. The end of a glass rod is immersed in a fluid culture of the test-organism and allowed to dry. While drying it is inserted into a sterile test-tube, and plugged around with cotton. It is then ready to test by exposure to any germicide, either liquid or gaseous. After exposure to the germicide it is plunged into a tube of sterile beef-broth in order to see whether the organisms adhering are all killed.

All of these methods are open to serious sources of error, particularly in the testing of powerful germicides. In method No. 1, small quantities of the substances tested may be carried over with the organisms, and, if a powerful germicide, in sufficient amount to prevent growth, and thus give erroneous results. In methods Nos. 2 and 3 this factor may be partially obviated by washing in sterile water after exposure to the germicide. This does not remove another source of error, namely, the chemical action that may take place between the substance and the protoplasmic contents of the bacterial cell. This action may extend deeply enough to restrain the growth of an organism for a very long time without actually killing it. When placed under suitable conditions, such union may be broken up and the organism regain its power to develop. It has been suggested that, to remove errors in the above methods, the bacteria after exposure to the germicide be inoculated into susceptible animals; but Sternberg's experiments in this direction have shown that bacteria may become so attenuated in virulence by the action of germicides insufficient to kill that the value of animal inoculation experiments is limited. Moreover, it sometimes happens that it is desired to test germicides on bacteria which are not pathogenic for animals.

Geppert suggested a valuable modification of these methods while determining the germicidal value of bichloride of mercury. After exposing his test-organism to bichloride of mercury, and before inoculating into bouillon to determine death of the organism, he treated with a dilute ammonium sulphide solution, thus effectually neutralizing any mercury-salt remaining.

Sedgwick developed this method still further, and to him we are indebted for demonstrating its accuracy and practicability. Sedgwick proceeds as follows:

4. To 15 c.c. of sterile water in a 60 c.c. Erlenmeyer flask add 2 c.c. of a virulent culture of the test-organism. Then add a solution of the substance under investigation in the proportion necessary to give the dilution wished. Mix thoroughly, and allow this "action-flask" to stand as long as it is desired to

have the germicide in contact with the test-organism (action-period). Transfer 0.5 c.c. from the action-flask to a flask containing 200 c.c. of a solution of some chemical capable of decomposing the substance being tested with the formation of inert or insoluble compounds. In this "inhibition-flask" the strength of the solution should be such that molecular proportions of the chemical are present in sufficient quantity to combine with all the germicides carried over. The inhibition-flask is shaken for thirty seconds, and 1 c.c. transferred from it to 100 c.c. of sterile water in another, the "dilution-flask." After two minutes, three agar tubes are inoculated with 1 c.c. each from the dilution-flask, plated, and growth watched for.

Control-experiments should be performed to determine that the dilution of the test-culture is not too great when carried through the three flasks. It likewise should be determined that the inhibiting chemical itself has no injurious effect on the bacteria.

The inhibiting chemical must be determined for each individual case. For salts of the heavy metals, ammonium sulphide answers well; for mercury-salts, stannous chloride may be used; for formaldehyde, ammonium hydrate; for carbolic acid, sodium sulphate.

The testing of gaseous disinfectants, such as sulphur dioxid and formaldehyde, should be conducted under conditions as nearly identical with those met with in actual practice as possible. The test-organisms may be exposed on threads or glass rods, and acted upon by a known volume of strength of germicide for a known length of time. Subsequent treatment of the organisms with a suitable inhibitor is necessary when possible and should growth occur in the cultures following, the test-organism should be identified in order to exclude possible contamination by extraneous organisms.

In determining the value of germicides for sterilizing ligatures, the students can apply methods based on the foregoing principles. Great care is necessary to arrive at correct conclusions, particularly in the case of animal tendons. In many instances quite stable compounds are formed between tendon and germicide, and living organisms may be so imbedded in such a substance that subsequent growth in a test-cuture is impossible. The use of a suitable inhibitor, and, prior to final culture-tests, a prolonged soaking in sterile water, will promote the accuracy of the results.

So many and often such obscure chemical and physical factors enter into the action of chemical germicides that uniform results are not possible within narrow limits. This accounts for the conflicting results obtained by different investigators, and even the same investigator at different times. A number of variable and only partially controllable conditions enter into every test. Results with gaseous disinfectants are especially uncertain on this account. No gaseous disinfectants have any great power of penetration, and consequently act only where the bacteria are freely exposed and then not always with certainty.

Physical Disinfectants.—*Drying.*—The effect of drying differs with different organisms and also with the same organism depending upon whether there are spores present or not. The vegetative cells of some organisms are very readily killed by drying, but spores, on the contrary, are not affected in this way. Spores have, in fact, been preserved for years dried on silk threads.

Agitation.—Bacteria are killed by prolonged violent agitation. They may be actually shaken to pieces. But ordinary gentle shaking appears in some cases at least to stimulate growth.

Cold.—Bacteria are very resistant to cold, those that have been so tested survive the low temperature of liquid air, though of course there is no growth at this temperature. The organisms subjected to this low temperature grow readily when inoculated upon culture media and removed to a suitable temperature.

Light.—Light is injurious to bacteria. Even diffuse day light is harmful, and direct sun light is distructive. Electric arc light is also injurious. The rays which are active are those at the ultra-violet end of the spectrum.

Heat.—Bacteria are very sensitive to heat. Even temperatures which are far short of burning or charring kill the bacteria very promptly. The vegetative cells are for the most part destroyed by temperatures around 60° C. in a comparatively short time,—ten or fifteen minutes. Spores are much more resistant to heat, some of these withstand the temperature of boiling water for a few minutes and some even for an hour or more.

Other Physical Agents.—Pressure, Röntgen rays, electric currents are not injurious to the bacteria.

CHEMICAL DISINFECTANTS.

The most efficacious method of disinfection is by heat applied as steam preferably under pressure. This mode of disinfection is applicable to bed clothing and underclothing, but it is not applicable to rooms and the like, and for this purpose recourse is had to various chemical agents. The list of substances possessing germicidal properties is large, but not all of these are available for practical disinfection. At one time the efficacy of chemical disinfectants was probably overrated. At all events, in practice much more drastic measures than would be indicated by laboratory experiments should be employed. In fact, the questions involved and the problems to be solved in practical disinfection are often very different

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*For fuller details on this subject consult Rosenau. Disinfection and Disinfectants. 1902. Also Gotschlich. Disinfection. Kolle and Wassermann. Handbuch der pathogenen Mikroorganismen. IV. Teil. 1, 1904. Harrington and Walker. *The Boston Medical and Surgical Journal.* April 23, 1903. Vol. CXLVIII., No. 17. Günther, *loc. cit.*

from the destruction of bacteria in laboratory experiments The disinfection of a cattle car or a Pullman sleeper is a very different matter from the killing of bacteria on silk threads or glass rods or bits of paper.

In the following pages only those substances which are in common use or seem to be of special value will be considered.

Verhoeff and Ellis* tested the following preparations for their germicidal power for S. pyogenes aureus: For one minute each, Liquor antisepticus, U. S. P., 100 per cent.; Listerine, 100 per cent.; Lysol, 1 per cent.; Cresylone 1 per cent.; Trikresol, $\frac{9}{10}$ per cent.; Acetozone, $\frac{1}{10}$ per cent.; Alphozone, $\frac{1}{10}$ per cent., and a number of other similar preparations in different strengths and for different lengths of time. They found that while they all killed the bacteria, none were more efficacious than the Liquor antisepticus. That any of the preparations act as intestinal germicides, as is claimed for Alphozone and Acetozone, is improbable from the fact that in albuminoid suspension the bacteria were found alive after twenty-four hours' action, of these agents.

Mercuric Chloride or Corrosive Sublimate.—This substance is probably more commonly used than any other one germicide. But Geppert whose work in this direction has been abundantly corroborated by others, found that the potency of corrosive sublimate as a germicide had been greatly overrated. The inhibitory action of corrosive sublimate, on the other hand, is very great, and the veriest trace of it left adhering to the bacteria is sufficient to prevent them from growing. Corrosive sublimate is difficult to remove by ordinary washing, traces of it remain even after very thorough washing. But if the last traces are removed by treatment with ammonium suphide or other reagents which precipitate the mercury-salt without themselves injuring the bacteria, growth takes place even where the corrosive sublimate solutions have been used which are

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Journ. Am. Med. Assn. V. XLVIII., No. 26. 1907. pp. 2175–2176.

apparently efficacious. Thus anthrax spores will not grow out in culture media when they are exposed for even a few minutes on silk threads to the action of corrosive sublimate solution of the strength of $\frac{1}{10}$ per cent. and then washed thoroughly in water and rinsed in alcohol; but Geppert showed that the spores so treated were only apparently killed, for it took twenty hours, exposure to corrosive sublimate solution of this strength where the spores were not dried on silk threads, but suspended in water, and where the last trace of corrosive sublimate was removed by treatment with ammonium sulphide. It is claimed that its affinity for albuminous bodies and the readiness with which it combines with such substances detract from its value for some purposes. On the other hand, many observers claim that the albuminous combinations formed under such circumstances are soluble in an excess of albuminous fluid, and that its value as a germicide is not affected thereby. To obviate this possible difficulty it is customary in practice to combine the bichloride of mercury with some substance that will prevent the precipitation of the mercury-salt by albumin. For this purpose 5 parts of any one of the following substances to 1 part of bichloride of mercury may be used—hydrochloric acid, tartaric acid, sodium chloride, potassium chloride, or ammonium chloride. A very practical stock-solution for laboratory purposes has the following composition:

Hydrochloric acid	100 c.c.
Bichloride of mercury	20 grams.

Five c.c. in a liter of water makes a solution of about 1–1000 strength.

Mercuric Iodide.—An extremely high antiseptic value has been placed on this substance by Miquel, who claims that the most resistant spores are prevented from developing in a culture-medium containing 1–40,000. In combination, as potassio-mercuric iodide, it has been used in soaps (McClintock) with very favorable results. The substance is not extensively employed, and further investigation is necessary to determine its true value.

Attempts are being made to manufacture combinations of mercury and other powerful metallic germicides with organic acid and basic bodies, the purpose being to utilize the metallic base in greater strength without injury to the living tissues. Such compounds are exemplified by *mercurol*, said to be a combination of mercury with nucleinic acid, and to possess active germicidal properties, great penetrating power, and no injurious effect on living tissue. It is also said to have a particularly destructive action upon the gonococcus.

Silver Nitrate.—This salt probably occupies the next position to the bichloride of mercury in germicidal power. Behring claims it to be superior to bichloride of mercury in albuminous fluids. The anthrax bacillus is killed by a solution of 1–20,000 after two hours' exposure. At least forty-eight hours' exposure to a 1–10,000 solution is required to kill the spores of anthrax. It is very irritating, and possesses strong affinities for chlorides, forming with them insoluble chloride of silver, a salt without germicidal value. For these reasons the use of silver nitrate is limited. In the solutions usually employed for douching the cavities of the body the available silver nitrate is immediately converted into the insoluble chloride, and little if any germicidal action takes place. To this fact may be ascribed the varying clinical results reported.

Many semi-proprietary silver compounds are on the market, introduced to replace the nitrate and its objectionable features. The most important are argentamin, argonin, protargol and argyrol, all organic silver combinations. They do not combine with chlorides, are less irritating than the nitrate and, not coagulating albumin, they possess greater penetrating power. Clinical reports and investigations have been so contradictory thus far that their value cannot be readily estimated.

Carbolic Acid.—One of the most important and most widely used germicides. It is usually employed in strengths of from 1 to 5 per cent. A 3 per cent. solution will sometimes kill the spores of anthrax after two days' exposure (see Bacillus anthracis, Part IV.). In the absence of spores the anthrax bacillus is destroyed by a 1 per cent. solution in one hour. The less resistant pus cocci are destroyed rapidly by a 2 per cent. solution. Combination with an equal proportion of hydrochloric acid enhances the efficacy of carbolic acid to a marked extent. This is due to the prevention of albuminous combinations, thus allowing greater penetration of the germicide.

McBryde* found that the admixture of lime to carbolic acid lessened the germicidal potency of the latter. He furthermore found that liquor cresolis comp., U. S. P., a liquid soap containing 50 per cent. cresol, miscible in all proportions with water, is a more potent germicide than carbolic acid. Liquor cres. comp. made from cresol of high boiling-points is more potent than that made of cresol of lower boiling-points. That made with cresol of 187 to 189° C. is nearly one and one-half times greater than carbolic acid.

Many other substances closely related to carbolic acid are used and possess marked germicidal properties. Among them may be mentioned creolin, cresol and lysol. They are all slightly superior to carbolic acid in actual germicidal value.

Aniline Dyes.—Many of these substances, notably pyoktanin (methyl-violet), possess germicidal properties. Malachite green is said to possess even greater germicidal value than pyoktanin. Methylene-blue also possesses considerable germicidal power.

Formalin is a 40 per cent. aqueous solution of formaldehyde. Results of the earlier investigations seemed to show that formaldehyde possessed remarkably high germicidal properties, but later experiments have failed to corroborate these. In solutions of 1–1000 an exposure of twenty-four hours is necessary

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*Dept. Agr., *Bu. An. In.*, *Bul.* 100. May 31, 1907.

to destroy the Staphylococcus pyogenes aureus, while 1–5000 is sufficient to restrain its growth (Slater and Rideal). Its use in a gaseous form as a house-disinfectant is by far the most important application at the present time.

In vaporizing the gas many methods have been employed. Simple evaporization of solutions without heat cannot be relied upon, for the solid, polymerized paraformaldehyde is easily formed under these circumstances. Better results can be obtained with the aid of heat, although polymerization is apt to occur unless evaporation is rapid. To produce the best results it has been found necessary to use special forms of lamps or generators for its production, a few of which are mentioned below.

Gotschlich* has carefully summed up the results obtained with formalin and its various preparations. He finds that while authorities agree in attributing very powerful inhibitory action to formaldehyde, they fail to get very powerful germicidal action. Even $\frac{1}{50000}$ shows marked inhibition, and $\frac{1}{10000}$ complete inhibition of bacterial growth in albuminous media even, varying of course with different bacteria. But in contrast to this 2.5 to 5 per cent. was required to kill the same organisms. The action of formalin is greatly increased by heating. Anthrax spores were killed in one and one-half hours in 12.5 to 15 per cent. formalin at room temperature, in the same solution they were killed in thirty minutes at 35° C., and in five minutes at 52° C. Lysoform, a perfumed soap containing formalin was found to be weak; septoform, a similar soap preparation was found to be somewhat more powerful; it has the remarkable property of being more strongly germicidal for anthrax spores than for the pus cocci. Other proprietary preparations were also found to be weak in germicidal power, others again more powerful. Acrolein which is allylaldehyde is said to have greater germicidal power than formalin.

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*Kolle and Wassermann. *Loc. cit*

A great many methods have been devised for using formalin for room disinfection in the form of vapor. These vary all the way from simply hanging up sheets wet with formalin to more or less elaborate lamps for burning methyl alcohol and converting it into formaldehyde. The two most important conditions to fulfill in the use of formaldehyde as a gas are temperature and moisture. The room to be disinfected should be warmed and the air saturated with moisture.

Hill* finds an explanation of the discrepancy in results obtained by different observers in the condition of the bacteria as to dryness or moisture, and concludes that for practical disinfection the destruction of bacteria thrown off from the patient in any way for some time before the disinfection of the premises should be aimed at.

Sanitary Construction Company's Lamp.—This lamp consists of a tank to hold the formaldehyde solution, and a spiral tube by which the solution is slowly conducted through a flame and vaporized. The necessary amount of solution is placed in the tank and the apparatus started, outside the room, the gas being conducted through the keyhole by a suitable tube.

Schering Lamp.—In this form of lamp formaldehyde is generated by the decomposition of paraform or paraformaldehyde, a polymeric modification of formaldehyde, occurring as a white salt. It is decomposed by heat, giving off formaldehyde gas. It is placed on the market in the form of tablets, each one of which yields a definite amount of gas. The lamp consists of a small iron tray for the reception of tablets, and so arranged above the heating apparatus that sufficient draught is created to carry off the gas as rapidly as formed. In operating, a sufficient number of tablets are placed on the tray, the lamp lighted and placed in the room to be disinfected.

**Journ. Infectious Diseases.* Supplement 2. Feb., 1906., p. 210.

Other references to formaldehyde in disinfection are:

Ravenel. Report of the American Public Health Association. Vol. XXVIII., p. 221.

Hill, Eben. Vol. XXIX., p. 208.

Rosenau. Disinfection and Disinfectants. Pub. Health and Mar. Hosp. Ser.

Evans. *Journ. Infec. Diseases.* Sup. No. 3, May, 1907.

Herzog. *Centralblatt f. Bakteriologie*, etc. Orig. XXXIV., 2, 1903.

Methyl-alcohol Lamps.—Several of these lamps are on the market, all operating on the well-known principle of the oxidation of wood-alcohol to formaldehyde when the alcohol is vaporized by projection against a heated, platinized, asbestos disk. In operating such an apparatus, the alcohol is lighted until the asbestos disk becomes hot. The flame is then extinguished; the heat from the disk is sufficient to vaporize the alcohol, which undergoes oxidation and keeps the disk at a red heat. When the apparatus is operating in a satisfactory manner, the room is closed and disinfection allowed to proceed. It must be said, however, that it is difficult to estimate or control the amount of formaldehyde evolved in generators of this type.

Formaldehyde Candles.—Mixtures of paraformalhyde and paraffin or other combustibles, which may be moulded into candles, each enclosed in a tin case, make a convenient apparatus to generate formaldehyde gas for room disinfection. The candle is placed in a suitable fireproof dish, it is then ignited, and generation of the gas is allowed to proceed in the tightly closed room.

Sulphur Dioxide.—This substance is used extensively for house disinfection, and is usually prepared by burning sulphur. Much difference of opinion exists regarding the value of it as a disinfectant. The spores of anthrax are not killed by several days' exposure to the liquefied gas. Anthrax and other bacilli are destroyed in thirty minutes when exposed on moist threads in an atmosphere containing one volume per centum of the gas. An exposure of twenty-four hours in an atmosphere containing four volumes per centum of the gas will destroy the organisms of typhoid fever, diphtheria, cholera and tuberculosis. The presence of moisture greatly enhances the activity of the disinfectant, owing to the formation of the more energetic sulphurous acid.

For the destruction of insects, such as mosquitoes, this agent is superior to formaldehyde. Its application for this purpose is important in preventing the spread of yellow fever and malaria.

In practice, at least three pounds of sulphur per 1000 cubic feet should be used, and moisture must be present. This latter requirement can be fulfilled by evaporating several quarts of water within the tightly closed room just prior to generating the gas. In using powdered or flowers of sulphur, the necessary amount is placed on a bed of sand or ashes in an iron pot, which should be supported on some bricks in a pan or other vessel containing an inch or two of water. The sulphur is ignited by means of some glowing coals, or by moistening with alcohol and applying a match. Difficulty is often experienced in keeping the sulphur burning, and for this reason it is surer and more convenient to use the so-called sulphur candles now on the market. In operating with these, a sufficient number are placed on bricks in a pan of water and the wicks lighted. Liquefied sulphur dioxide may be used, and can now be obtained in convenient tin receptacles containing a sufficient quantity for the disinfection of an ordinary room. The can is opened by cutting through a soft metal tube projecting from the top. The fluid vaporizes at the room temperature, and it is simply necessary to place the pan in a convenient porcelain dish and allow the fluid to evaporate.

Sulphur dioxide is objectionable on account of its lack of power when dry, and on account of its corrosive action on metal and its bleaching effect on hangings and draperies in the presence of moisture; it is, therefore, preferable to use formaldehyde when possible.

Chlorine.—A very active gaseous disinfectant, particularly in the presence of moisture. An atmosphere containing 1 per cent. of the dry gas is fatal to anthrax spores in three hours. The anthrax bacillus is killed in twenty-four hours by exposure to a moist atmosphere containing the gas in the proportion of 1–2500. The bacillus of tuberculosis is killed by an exposure of one hour to a moist atmosphere containing the gas in the proportion of 1–200. Extremely minute quantities in solution will prevent the development of putrefactive organisms. The substance has been used for house and ship disinfection, but is now seldom employed on account of its extremely irritating properties and the difficulty of handling.

Bromine.—Used in the gaseous and liquid form. The dry vapor possesses but little disinfectant power; when moist it is much more efficient. In saturated acqueous solution it will kill the anthrax bacillus in twenty-four hours.

Calcium Hypochlorite, usually known as Chloride of Lime.—This is a most practical and valuable disinfectant, depending for its efficiency on the available chlorine contained in it. Its alkalinity favors penetration, and for many purposes it cannot be excelled. A 1 per cent. solution will destroy anthrax spores in one hour. A solution of the same strength will disinfect typhoid stools in ten minutes.

Lime.—The addition of 0.1 per cent. of unslaked lime to fluid-cultures of the typhoid bacillus and cholera spirillum will render them sterile in four or five hours. Typhoid dejecta are sterilized in six hours by the addition of 3 per cent. of slaked lime; the addition of 6 per cent. will accomplish the same result in two hours. A convenient form for practical use is an aqueous mixture containing 20 per cent. of lime—so-called milk of lime. Typhoid and cholera dejecta are sterilized in one hour after the addition of 20 per cent. of this mixture. In practice it is safer to use a considerable excess of lime. From the foregoing facts it would seem probable that lime or whitewash as ordinarily applied would possess disinfectant properties. Experimental work has demonstrated this to be a fact. The organisms of anthrax, glanders and the pus cocci were destroyed within twenty-four hours by one application. For spore-forming organisms and the bacillus of tuberculosis the power is not so great, the latter organism not being destroyed by three applications of the whitewash. This is due, perhaps, to the large amount of fatty matter in the bacillus of tuberculosis, and suggests the possibility of enhancing the efficacy of the lime by the addition of a small proportion of caustic alkali.

Hydrogen Peroxide.—This substance is placed on the market in solutions varying in strength from 10 to 30 volumes; the mode of expression indicating that corresponding solutions will liberate ten to thirty times their volume of oxygen when appropriately treated. It possesses the property of rapidly oxidizing purulent secretions, and on this account is much used for cleansing infected wounds. It deteriorates in strength so rapidly that only fresh solutions of known strength should be used.

Potassium Permanganate.—Koch asserts that a 3 per cent. solution will destroy anthrax spores in twenty-four hours, but that a 1 per cent. solution cannot be depended upon to kill pathogenic organisms. Its disinfectant value in practice is very low on account of its ready decomposition by inert material. In the dilute solutions usually used for medicinal injections and irrigations no disinfectant action occurs.

Iodoform.—This substance possesses little if any disinfectant power. It is mildly antiseptic in moist wounds, due to the gradual liberation of small quantities of iodine.

Boric Acid.—This material possesses practically no disinfectant power. It is a mild antiseptic when applied as an undiluted powder to wounds. A saturated aqueous solution is much used, and is weakly antiseptic.

Essential Oils.—Many of these bodies possess germicidal value, notably the oils of cinnamon and cloves. The oil of mustard is also a valuable disinfectant, but so irritating that the pure oil cannot be used. The use of powdered mustard in the autopsy-room will remove the foul odor from the hands more rapidly and completely than any other means.

Coal Oil or Petroleum.—While the disinfectant value of this substance is slight, its use in destroying the larvæ of insects, such as the mosquito, has given it an important position in preventing the spread of malaria and yellow fever. A small amount poured on a stagnant pool rapidly spreads over the surface and effectually destroys such larvæ.

Ferrous Sulphate (Copperas).—This salt has been much used, but possesses only feeble disinfectant powers. A 3 per cent. solution requires three days to kill the bacillus of typhoid fever. On account of its affinity for ammonia and sulphides it is an efficient deodorizer for temporary use, but cannot be relied upon to kill the bacteria producing the noxious gases.

Cupric Sulphate (Blue Vitriol).—This salt is quite an efficient disinfectant. In a solution of 1–3000 the spirillum of cholera is destroyed in ten minutes. A 5 per cent. solution will kill the typhoid bacillus in ten minutes. A solution of from 2 to 3 per cent. in strength can be relied upon to destroy all pathogenic organisms that do not form spores.

Zinc Sulphate.—This salt is a very feeble disinfectant. Pus cocci are not destroyed in two hours by a 20 per cent. solution. As a deodorizer it has about the same value and acts in the same way as ferrous sulphate.

Zinc Chloride.—A 2 per cent. solution will kill pus cocci after an exposure of two hours. It is therefore a much more powerful disinfectant than the sulphate.

Disinfection of the Mouth.—Wadsworth* found that for mouth disinfection in pneumonia, of all the antiseptics in common use alcohol alone proved efficient when tested on the pneumococcus He tested a long list of various preparations, but these were all counteracted by the mucus in the mouth.

**Journ. Infectious Diseases.* Vol. III., p. 774.

Disinfection of Dejecta and Urine.—A 4 per cent. solution of calcic hypochlorite (chloride of lime) is most efficient and rapid for this purpose. A convenient solution contains six ounces of the salt to one gallon of water. The excreta should be received in a suitable vessel and immediately mixed with an equal bulk of the disinfectant. The contents of the vessel should be allowed to stand for one hour before emptying. A 20 per cent. milk of lime is just as efficient, and possesses the advantage of cleanliness and lack of odor. It should be used in the same quantity and allowed to act for the same length of time. A 5 per cent. solution of carbolic acid may be used, but should be allowed to act for at least four hours.

Disinfection of Sputum.—The chemical disinfection of tuberculous sputum is somewhat difficult on account of the large amount of albumin in it and the fatty matter associated with the bacillus of tuberculosis. Dilute solutions of bichloride of mercury are apt to be decomposed and rendered inert by the albumin. Carbolic acid is open to the same objection, but its combination with hydrochloric acid can be used successfully in a strength of 5 per cent. each. Milk of lime cannot be relied upon for this purpose. A 4 per cent. solution of calcic hypochlorite (chloride of lime) is the best for general use, and the spit-cup should be kept nearly full of this solution. Sputum may also be disinfected by exposure to the action of steam in the sterilizer or by boiling for fifteen minutes. If napkins or old pieces of cloth are used for the reception of sputum, they may be immediately destroyed in a fire.

Disinfection after Postmortems.—After autopsies on infectious cases it is necessary to disinfect the table and fluid products coming from it prior to emptying into the sewer. The table may be disinfected by a liberal sprinkling with 4 per cent. calcic hypochlorite solution. All fluids should be treated with an equal quantity of the same solution. The table should not be cleaned for at least one hour after application of the disinfectant. The same rule applies to the disinfection of the fluids—an exposure of at least one hour to the disinfectant before final disposition.

The Cadaver in Contagious Diseases.—In cases of death from a contagious disease all the orifices of the body should be packed with cotton soaked in a strong solution (1–500) of bichloride of mercury, the skin washed with a 1–1000 solution, and the cadaver wrapped in a sheet wet with the same. The funeral should be private and the body disposed of within twenty-four hours, preferably by cremation.

House Disinfection.—After infectious disease it is essential that the house or the apartment in which the patient has been confined should be disinfected. It is rarely necessary to carry out the process in more than two rooms; but should it be so, the process can be applied to the whole house.

After thorough bathing of the patient, preferably with an antiseptic soap, the individual should be wrapped in a clean sheet and removed to a clean room. All articles or materials that are of little value should be destroyed. All bedding, towels and the like should be placed in wooden tubs and covered with a 1–1000 solution of bichloride of mercury. The room should then be made as nearly airtight as possible; this can be accomplished by pasting strips of paper over registers, cracks, spaces between window-sashes and the like. Formaldehyde gas is then passed through the keyhole into the room (or it may be generated by formaldehyde candles) in sufficient quantity to destroy the infectious element.. The room should be sealed for at least twelve hours, after which time it may be opened and aired. The process is completed by washing all exposed surfaces in the room with 1–1000 bichloride of mercury. This latter requirement is not essential if the gaseous disinfection has been complete, but since we have no absolute knowledge on this point, the secondary washing should be carried out. This method can be considered reliable for surface disinfection, but for the interior of mattresses and stuffed furniture-cushions it is not certain. In all cases where absolute disinfection is demanded, such articles must be ripped apart and loosely exposed to the gas or sterilized by steam under pressure. Instead of formaldehyde, sulphur dioxide may be used for room disinfection, but in the light of present knowledge the formaldehyde method is superior.

Stokes and Stubbs* found that reinfection of premises which had been disinfected with formalin occurred in the 2807 cases of diphtheria which were made the subject of inquiry in 2.35 per cent. in the same year: in the 2739 cases of scarlet fever similarly examined reinfection of the premises occurred in 2.55 per cent.

*W. R. Stokes and W. P. Stubbs. *Maryland Medical Journal.* Feb., 1907.

CHAPTER VIII.

SURGICAL ANTISEPSIS.

Many important advances in surgery have been made possible by the practical application of the principles of sterilization and antisepsis set forth in the preceding chapter. Previous to the introduction of antisepsis in surgery there was imminent risk of suppuration and septicemia even in the most insignificant operations, and many of the capital operations which are now daily performed without serious risks were at that time so often attended by fatal consequences due to infection that the patient and the surgeon were deterred from taking the chances.

The four sources of infection in surgery are from the bacteria in the air of the room in which the operation is performed, the bacteria on the surface of the patient's body, the bacteria on the hands of the surgeon, and finally the bacteria on the instruments with which the operation is performed and on the suture material and dressings. All of these sources of infection have to be taken into account and guarded against.

In the modern operating-room the danger from infection through the air is reduced very greatly. The room is so constructed and fitted with tables and other necessary furniture that everything may be washed and rubbed with antiseptic solutions. Everything is done to avoid the accumulation of dust and to prevent the distribution of dust in the air. The walls and floor are wiped with moist cloths since the bacteria cannot be blown from moist surfaces by any ordinary circulation of the air nor by even any draught that is ordinarily encountered in an operating room. On the contrary the bacteria become very easily disseminated through the air where everything is dry. Even walking through the room is sufficient to stir up the bacteria from a dry, dusty floor. The air is, however, probably not as serious a source of danger as the others mentioned, for there are not as many bacteria, specially not as many pathogenic bacteria found in the air as was at one time supposed to be present there. In the early days of antiseptic surgery the air was supposed to be such a dangerous source of infection that operations were performed under a carbolic acid spray. The seat of operation and the hands of the operator were sprayed continuously throughout the operation. This practice is no longer employed. Still the danger from the air infection although less serious than was at one time supposed should nervertheless not be overlooked.

The danger from the introduction of bacteria into a surgical wound from the surface of the patient's own body must be carefully guarded against. There are apt to be pus-producing cocci on the surface as well as deeper in the layers of the epidermis. The staphylococcus epidermidis albus (Welch) is found to be quite constantly present in the deeper layers of the epidermis, and is apt to give rise to the stich abscesses which so often form around sutures in even the most successful operations. Surgical cases also are common in which bacteria have been introduced in an accidental wound either at the time of the accident, as where the patient has received an accidental wound with some infected instrument, or where the patient has been thrown to the ground and had dirt rubbed into the wound, or where the patient has been wounded with a dirty splinter of wood or dirty nail or the infection of the wound may take place later by the dressing of the wound with dirty rags. In every case the seat of operation should be disinfected as thoroughly as possible. Unfortunately this is a difficult, not say an impossible task in some case at least. Certain parts of the body are much more easily disinfected than other parts. The abdomen is comparatively easily disinfected, but the epidremis of the hands is very difficult. The mucous membranes are difficult to disinfect for the reason of there delicate structure, the interference of the secretions with the action of disinfectants which precipitate them, and moreover because the disinfectants have so little power of penetration below the surface. The lack of penetration also interferes with the action of disinfectants on the epidermis. The application of disinfectants to the surface does little good unless it is preceded by thorough scrubbing with soap and water. The mechanical washing is of more importance than the application of disinfectants in any case. In fact some have advised the use of scrubbing with soap and water alone, but it would seem rational to suppose that the additional use of disinfectants would be of advantage.

It follows from what has been said that it is very difficult for the operator to sterilize his hands, or at least to be sure that the hands are sterile. Many different methods have been recommended for the purpose, all of them having this in common that they recognize the importance of thorough scrubbing with soap and water, with particular attention to the folds of the epidermis around the nails and under the nails. All seem to agree that the subsequent treatment of the hands with alcohol as advised by Fürbringer, no matter what subsequent treatment is employed, is very efficacious. The strength of alcohol recommended is at least 80 per cent., and the hands should be washed in the alcohol for a few minutes. After the scouring with soap and water and washing in alcohol, some advise no further sterilization, others prefer to use various antiseptics. The alcohol has not only germicidal value, but it also frees the hands from grease and thus prepares the skin for treatment with antiseptics. But it has been pointed out that the adjuvant action of alcohol when used along with other antiseptics cannot be attributed to its solvent action on the fat alone, for ether is a better solvent for fat than alcohol is, and yet ether has been found not to aid the action of antiseptics on the hands to the same extent as alcohol. What has just been stated in regard to the sterilization of the hands of the surgeon applies, of course, also to the field of operation.

The use of rubber gloves and of various other impervious coatings for the hands are extensively employed, and the rubber gloves give excellent results where it is feasible to use them.

The various coatings which have been recommended from time to time seem not to have found general adoption for the reason that all of them soon crack or wear off during the operation. The use of sterilized rubber gloves has the double advantage of lessening the chances of infecting the patient and of insuring the operator against infecting himself when handling wounds which are already infected, as in the case of syphilitic infection. Rubber gloves may be sterilized by steam or by the use of disinfecting solutions. Ordinary cotton gloves seem also to afford a considerable degree of protection for the patient and the operator.

Materials used for sutures and dressings are best sterilized by steam, preferably in an autoclave. All sutures may be sterilized with steam without injuring them except catgut which swells up and softens and becomes useless when treated in this way. Catgut is of such great value as a suture material particularly for deeply buried sutures and in abdominal surgery on account of the fact that the catgut does not have to be removed, but is absorbed by the tissues that every effort has been made to devise a sure method for its sterilization. Consisting as is does of the animal intestine it is apt to contain pathogenic bacteria the colon bacillus at least derived from the feces with which it was in contact, and is to be regarded as essentially infectious in itself. So far no certain means of sterilization have been devised for catgut, though very many experiments have been made to accomplish it. The methods which seem to have geven the best results all consist in subjecting the gut to high temperatures in alcohol or some heavy oil which does not materially injure its structure. Some surgeons, however, have reluctantly abandoned the use of catgut altogether on account of the difficulty of sterilizing it. Silver wire is a valuable material for sutures for the reason that it may be easily sterilized by heat without injuring it, and moreover silver is itself a potent germicide. Stitch abscesses seldom or never form around silver wire sutures.

For metallic instruments boiling for five minutes in 1 per cent. soda solution is said to suffice amply for thorough sterilization. This does not dull the edge provided the instruments are supported on some kind of rack above the bottom of the vessel in which they are sterilized. But they should be properly constructed in the first place, and should consist entirely of metal. Scissors, hemostats, syringes and the like should be so constructed that they are easily taken apart.

Brushes used for scouring the hands and the field of operation are difficult to sterilize with certainty. The best method seems to be to sterilize them in the autoclave and lay them in a disinfecting solution till needed.

Rubber catheters are very hard to sterilize. The best way seems to be to keep them in corrosive sublimate solution 1–1000, and to be sure that the disinfectant fills the lumen as well as coming in contact with the outside.

Sterilization of solutions used in subcutaneous injections is attended with some difficulty. Some of the chemicals of which these solutions are made are injured by heat. Various suggestions have been made to render such injections less liable to produce infection. Making up the solutions with 1–10000 corrosive sublimate is advised, also keeping the drugs dissolved in strong alcoholic solutions, and diluting with sterilized water as needed. Various infections have been from time to time reported from the use of subcutaneous injections of contaminated solutions.

PART III.

NON-PATHOGENIC BACTERIA.

No very sharp line can be drawn between pathogenic bacteria on the one hand, and non-pathogenic bacteria on the other; for many kinds of bacteria which occur as saprophytes under ordinary circumstances may multiply in the body and cause injury under certain conditions. Bacillus prodigiosus, an organism which is given to students in the beginning of their studies in bacteriology, on account of its harmlessness, produces lesions in experiment animals when injected into the peritoneal cavity. But those organisms may be fairly classed as non-pathogenic which are not found to cause spontaneous disease, and which do not cause disease in animals when in troduced in small amounts.

The number of species of non-pathogenic bacteria is very large. Eisenberg* describes 376 species of bacteria, mostly non-pathogenic. Sternberg† enumerates 489 species, including the pathogenic varieties; but the majority, of course, are nonpathogenic. Flügge‡ considers about 500 species of bacteria. Migula§ recognizes nearly 1300, and Chester|| about 800 species. Probably some of the bacteria which have been described as distinct species are in reality not different; but, on the other hand, it is also probable that a still larger number of spices have not been described at all—how many, it is impos-

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Bakteriologische Diagnostik. 1891.
†Manual of Bacteriology. 1893.
‡*Die Mikroorganismen.* 1896.
§*System der Bakterien.* 1900.
||Manual of Determinative Bacteriology. 1901.

sible to say. In a work of this character it is feasible to mention only a few of the commonest and best-known species of non-pathogenic bacteria or such as have some marked peculiarity.

Micrococcus Agilis.—Found in water; coccus about 1 μ in diameter, usually appearing as diplococci, sometimes as streptococci and tetrads; liquefies galatin slowly; grows at room temperature, on ordinary culture media, forming a rose-red pigment on agar and potato. This micrococcus is remarkable in being actively motile; it possesses a flagellum. It is stained Gram's method.

Micrococcus Ureæ.—Found in decomposed, ammoniacal urine and in the air; coccus, 0.8 to 1 μ in diameter, occurring singly or in various combinations; does not liquefy gelatin; facultative anaerobic; grows rapidly, best at 30° to 33° C.; grows on ordinary gelatin, but best on special media; it decomposes urea, producing ammonia and carbon dioxide, which form ammonium carbonate.

Sarcinæ.—There is a large number of species of sarcinæ. They are common organisms in the air. They frequently contaminate plate-cultures. Many of the sarcinæ of the air present, in cultures, growths having brilliant colors, from which some of them are named; thus there are arange, yellow, rose-colored and white sarcinæ, and others.

Sarcina Pulmonum.—Found in the air-passages of man; 1 to 1.5 μ in diameter, occurring in tetrads or cubes of eight cells; aerobic; does not liquefy gelatin; grows slowly, best at ordinary temperature, preferably upon gelatin. It decomposes urine with the formation of ammonia. It is said to form endogenous spores which are extremely resistant toheat.

Sarcina Ventriculi.—Found in the stomachs of man and of animals; 2.5 μ in diameter, occurring in cubes of eight cells or more; it does not liquefy gelatin; aerobic: grows on ordinary culture-media; the growths tend to become yellow. Small numbers of sarcinæ may occur in the normal human stomach; the presence of large numbers indicates the existence of abnormal fermentative processes.

Bacillus Fluorescens Liquefaciens.—Found in water and putrid fluids; very common; appears as a small rod, actively motile; aerobic, but somewhat variably; liquefies gelatin; grows rapidly at ordinary temperatures upon the usual culture-media. It forms a pigment having a beautiful greenish-yellow fluorescence, best seen in transparent media; the growth on potato has a brown color. Does not stain by Gram's method and does not form spores.

Bacillus Fluorescens Putidus.—Found in water; a short rod with rounded ends; actively motile; does not liquefy gelatin; aerobic; does not form spores; grows rapidly at the ordinary temperatures upon the common media. Gelatin cultures give off a powerful, foul odor of trimethylamin. It produces a greenish, fluorescent pigment, best seen in transparent media; on potato the growths form a thin, gray to brown, slimy layer.

There are several other fluorescing bacilli, mostly found in water.

Bacillus Indicus.—Found by Koch in the stomach contents of an ape in India; a fine short bacillus with rounded ends; motile; does not form spores; facultative anaerobic; liquefies gelatin; grows rapidly, best at 35° C. upon the ordinary media; produces a brick-red pigment. Very large doses injected into rabbits caused death in three to twenty-four hours.

Bacillus Prodigiosus.—Widely disseminated in the atmosphere of certain places; a short bacillus with rounded ends, in form often nearly like the micrococci; facultative anaerobic; not motile, as a rule; does not form spores; liquefies gelatin rapidly; grows rapidly, best at 25° C. on the ordinary culture-media; milk is coagulated; gas forms in sugar-media; cultures on potatoes give off a foul odor of trimethylamin. A brilliant red color, which develops only in the presence of oxygen, appears in cultures. The pigment appears as granules outside of the bacteria.

Bacillus Violaceus (of Berlin).—Found in water; a slim rod with rounded ends which may form threads; actively motile; facultative anaerobic; liquefies gelatin rapidly; forms endogenous spores placed near the centers of the bacilli; grows rapidly, and not at high temperatures, upon ordinary media, forming a deep, violet-colored pigment. There are several bacilli related to this one.

Bacillus Amylobacter (Clostridium butyricum; Bacillus butyricus, Prazmowski).—Found widely distributed in nature in decomposing vegetable material and in the stomachs of ruminant animals; a large, thick rod with round ends, often arranged in chains; actively motile; anaerobic; forms spores which are located in the center of the bacillus and give it a spindle-shaped form, or at one end, when it has the outline of a tadpole; has not been cultivated satisfactorily on ordinary media; grows best at 35° to 40° C.; decomposes carbohydrates with the formation of butyric acid; decomposes cellulose. Organisms of similar form have been found as fossils belonging to the carboniferous period.

Bacillus Butyricus (Hueppe).—Found in milk; appears as a small, irregular rod, also forming threads; very actively motile; aerobic; rapidly liquefies gelatin; forms centrally located spores; grows best at 35° to 40 C.; grows rapidly on ordinary media; coagulates milk, redissolving the coagulum, producing also butyric acid. A large number of bacteria, both aerobic and anaerobic, produce butyric acid fermentation.

Bacillus Megaterium.—Obtained by de Bary from cooked cabbage-leaves; common on plants and earth; a large bacillus with rounded ends, often forming chains; motile; slowly liquefies gelatin; aerobic; forms spores, especially in potato cultures; grows rapidly at room temperature on the ordinary media.

Bacillus Mesentericus Vulgatus (Potato bacillus).—Found on potatoes; common in earth; a large, long rod with rounded ends, often forming long chains; motile; it is stained by Gram's method; liquefies gelatin; aerobic; forms spores; grows rapidly, best at about 20° C.; grows on ordinary media, forming on potato a thin, wrinkled membrane which spreads rapidly over the surface. It coagulates milk, redissolving the coagulum. It possesses numerous flagella. The spores are extremely resistant to heat.

Bacillus Phosphorescens Indicus.—Obtained from sea-water; a small, thick, rod-shaped bacillus with rounded ends, also forming threads; actively motile; not stained by Gram's method; liquefies gelatin; aerobic. It grows slowly, best between 20° and 30° C., upon the usual media, except milk and potato. Its culture, when old, especially when on animal nutrient-media and in the presence of certain sodium salts, are phosphorescent in the dark.

There are various other bacilli which produce phosphorescence, some of which do not liquefy gelatin.

Bacillus Mycoides (Bacillus ramosus; W*urzelbacillus*).—Found in the earth and in water; very common; a large, short bacillus with rounded ends, often forming chains and threads; slightly motile; liquefies gelatin; aerobic forms centrally located, oval spores; grows rapidly at room and incubator temperatures upon the usual media. It is said rapidly to decompose albumin with the formation of ammonia.

Bacillus Subtilis (Hay bacillus).—Found on hay, in the air, water, ground and decomposing fluids; very common; a large bacillus somewhat resembling the anthrax bacillus in form, with rounded ends, often forming chains or long filaments; motile; possessing flagella; liquefies gelatin; aerobic; it is stained by Gram's method. It may have large, centrally located spores, which form best on potato at about 30° C. The spores are extremely resistant to heat and to chemical germicides. It grows best at about 30° C. upon the ordinary culture-media; milk is peptonized. Bacillus subtilis may easily be isolated in pure culture by adding finely cut hay to tubes of bouillon; placing these in the steam sterilizer for five or ten minutes; then letting the tubes develop in the incubator. Plates made from the bouillon will probably show colonies of the Bacillus only, as the steam may be expected to have destroyed all organisms except its very resistant spores.

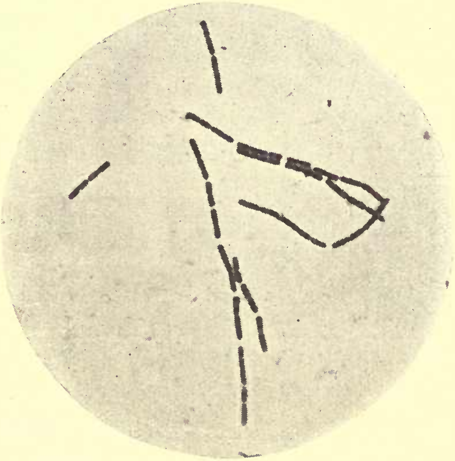

FIG. 59.—Bacillus subtilis. (× 1000.)

The hay bacillus has certain congruers, and it is perhaps more correct to speak, as is often done, of the "hay bacillus group" rather than of a special organism. Some of the congruers have been found in pure culture in cases of panophthalmitis following injury. Moreover, injections of cultures of the organism so obtained, produced panophthalmitis in experiment animals.*

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*Silberschmidt. *Ann. de l'Institute Pasteur.* 1903. p. 268. Also see Kneass and Sailer. *Univer. Pennsylvania Med. Bull.* June, 1903.

Bacillus Erythrosporus.—Found in decomposing fluids and water; a slim bacillus with rounded ends; motile; does not liquefy gelatin; facultative anaerobic; forms oval, red-colored spores, two to eight in each filament; grows rapidly, only at ordinary temperatures; produces a greenish-yellow, fluorescent pigment. On potato it forms a limited, reddish growth becoming nut-brown.

Bacillus Cyanogenus (Bacterium syncyanum; Bacillus lactis cyanogenus; Bacillus of blue milk).—A bacillus of variable size, with rounded ends; motile; spore formation doubtful; is aerobic; not stained by Gram's method; grows rapidly at ordinary but not so well at incubator temperatures on the usual culture-media; does not liquefy gelatin; produces a grayish-blue pigment, brighter in acid media, at ordinary temperatures; milk is not coagulated or rendered acid.

Bacillus Acidi Lactici (Hueppe).—Found in sour milk; a short, plump rod; not motile; does not liquefy gelatin; facultative anaerobic; grows on the ordinary media; in milk causes development of lactic acid with precipitation of casein and production of gas and alcohol. It belongs in the same group as B. coli communis and B. lactis aerogenes (see Part IV.).

There are numerous other bacteria, such as the Bacterium acidi lactici, which cause the formation of lactic acid in milk.

Heinemann,* as the result of his studies, comes to the conclusion that it is not justifiable to regard B. acidi lactici as a specific bacillus.

Bacterium Ureæ.—A short, thick bacillus with rounded ends; not motile; aerobic; found in ammoniacal urine; grows slowly at room temperature upon gelatin, which is not liquefied; decomposes urea; forms ammonium carbonate.

Bacterium Zopfii.—Found in the intestines of hens, in water and in fecal matter; a bacillus 0.75 to 1 μ broad and 2 to 5 μ long; may form threads. Actively motile; does not liquefy

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*Heinemann. *Jour. Inf. Diseases.* Vol. III., p. 173.

gelatin; aerobic; involution forms are often seen and they have been described as spores; grows rapidly, best at 20° C. upon gelatin; forms branching zoöglœæ. It is a member of the same group as B. proteus (see Part IV.).

Spirillum Rubrum.—Found by Esmarch in the putrefying cadaver of a mouse; short spirals twice the breadth of the cholera spirillum, usually with one to three turns; in bouillon growing into long spirals; motile, with flagella; spore formation doubtful; facultative anaerobic; does not liquefy gelatin; grows slowly, best at about 37° C. on the ordinary media; produces a wine-red pigment only when the air is excluded.

Spirillum or Spirochæta Dentium.—Found in the mouths of healthy persons, on the margins of the gums when they are covered with a dirty deposit; long spirals with several windings, uneven in thickness; has not been cultivated.

Spirillum Sputigenum.—Found in the human mouth in healthy persons at the margin of the gums; curved rods or short spirals which resemble the spirillum of cholera in form; has not been cultivated.

Spirillum Rugula (Vibrio rugula).—Found in swamp water, in fecal matter and in the tartar of the teeth; a curved rod 0.5 to 2.5 μ broad and 6 to 8 μ long, having one flat spiral winding; motile, with flagella at the ends; probably anaerobic; forms spores located at the ends.

Spirillum Volutans.—Found in swamp water; very long spirals with several turns; 1.5 to 2 μ broad and 25 to 30 μ long; motile, with a flagellum at each extremity. The protoplasm is granular.

Spirillum Undula.—Found in putrefying infusions containing organic matters; a rather short spiral form with three turns or less, about 1 μ thick and 8 to 12 μ long; actively motile, with a tuft of flagella at each extremity; has been cultivated on agar.

Spirillum or Spirochæta Plicatile.—Found in swamp water; spiral forms of various lengths; sometimes 100 to 200 μ long; actively motile.

The spirilla (vibrios or comma-shaped forms) closely resembling the spirillum of cholera, will be considered in connection with that organism.

Fusiform Bacillus.—Vincent* was the first to describe fusiform bacilli which he isolated from a case of diphtheroid angina, and since his publication his observations have been more or less corroborated by a number of others.

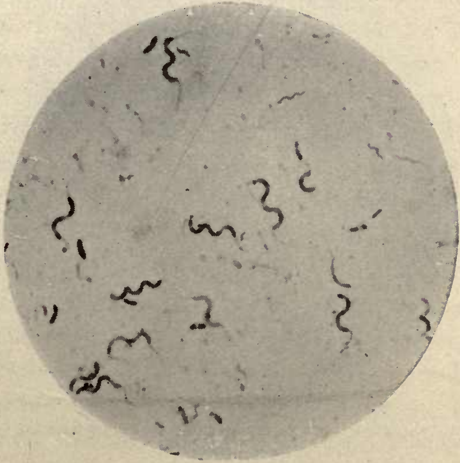

FIG. 60.—Spirilla from swamp water. (× about 500.)

Weaver and Tunnicliff† cultivated fusiform bacilli from a case of ulceromembranous stomatitis and from a case of diphtheria. The cultures were grown under anaerobic conditions at 37° C. They were inclined to regard the spiral forms always present along with the fusiform organism as different from these, but upon further observation Tunnicliff‡ showed

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***Ann. de l'Inst. Pasteur.* 1899. p. 609. (Cited from Günther. *Loc. cit.*)

†Weaver and Tunnicliff. *Journal Infectious Diseases.* Vol. II., 1905. pp 446–459. Also Ebenda. Vol. IV., No. 1. pp. 8–33.

‡Tunnicliff. *Journ. Infectious Diseases.* Vol. III., 1906. pp. 148–155.

that the spiral forms are in reality not separate organisms but represent stages in the cycle of development of the fusiform bacillus which is polymorphous to a very marked degree, showing short bacilli, long filaments and spirals. The bacilli form spores, usually one spore to each bacillus, sometimes two. The filaments are either straight or wavy. The spores retain the stain when treated as in staining tubercle bacilli and decolorized with 1 per cent. sulphuric acid. The cultures upon which Tunnicliff's observations were made were obtained from three normal throats, but the culture corresponded in all respects with those obtained by her and Weaver already mentioned.

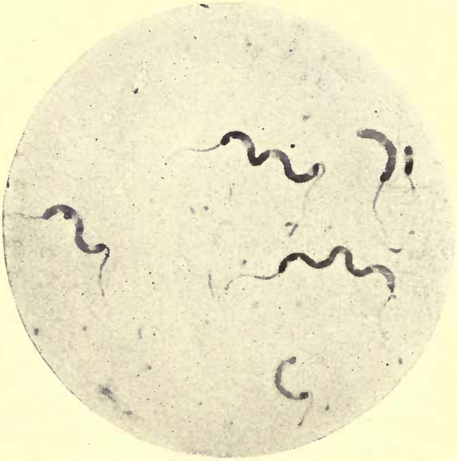

FIG. 61.—Spirilla from swamp water showing flagella (Löffler stain). (X 1000.)

Higher Bacteria.—Certain organisms (beggiatoa, thiothrix leptothrix, cladothrix, actinomyces or streptothrix) of more complicated structure than most bacteria, but resembling them in many respects, are called "higher bacteria." They consist of definite filaments which are usually made up of rod-shaped elements, but the relation between these elements is very intimate.

Some of them (beggiatoa, thiothrix) contain sulphur granules. Many of them occur in water. There are forms among them which are found attached to some object by one end of the filament (thiothrix). Some of them (actinomyces or streptothrix) have branching filaments, which are rarely seen among the lower bacteria (see page 119). Often one end of the filament becomes specialized for the purposes of reproduction. The fungus of actinomycosis is the best known of this group. There are many other members, however, both pathogenic and non-pathogenic. Most of them require still further study. The tubercle bacillus and other acid-proof bacilli which resemble it have some points of resemblance with actinomyces (see B. tuberculosis, Part IV.).

Leptothric Buccalis.—Found in the mouth cavity. This name has been applied to large, twisted, thread-like organisms, in which segments can be demonstrated with difficulty or not at all. Apparently, different organisms have been described under this name. Vignal claims to have cultivated a Leptothrix buccalis. Miller recognizes two principal species, neither of which could be cultivated—*Leptothrix innominata*, which shows no transverse divisions, and which is stained faintly yellow by iodine; and *Bacillus buccalis maximus*, in which the transverse divisions are distinct, and which is stained brownish-violet by iodine. Miller's *Leptothrix maxima buccalis* is similar to the last except in lacking the iodine reaction.

A variety of leptothrix, or a nearly related organism, appears to be the most frequent cause of the form of gangrenous inflammation of the mouth and genitals called noma. It stains faintly by Gram's method. It does not grow on ordinary media.* Another organism of this group has been described which is pathogenic to a number of domestic animals.†

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*Blumer and MacFarlane. *American Journal Medical Sciences.* November, 1901.

†It has also been called "necrosis bacillus," and "Streptothrix cuniculi." Pearce. *University of Pennsylvania Medical Bulletin.* November, 1902.

Yeasts and Moulds.—In the course of bacteriological work one constantly encounters yeasts and moulds, which, although not bacteria, must nevertheless be understood and recognized to avoid error. Accidental contamination of tubes or plates is likely to be the result of the growth of some of these forms. The yeasts generally go by the name of *saccharomyces*, of which there are several spices. The *Saccharomyces cerevisiæ* is the ordinary yeast of alcoholic fermentation. Some of the yeasts present colored growths—red, white and black. They consist of large, oval cells, which readily stain with the aniline dyes. They multiply by the protrusion of a little bud from the cell, which develops into a new cell. In an actively germinating growth of yeast these budding cells are readily distinguished (Fig. 62).

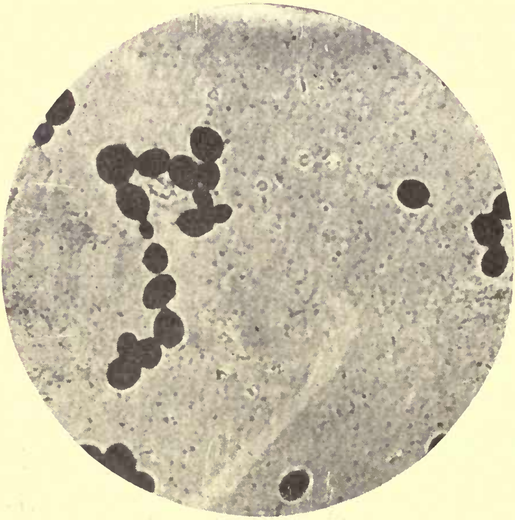

FIG. 62.—Yeast cells stained with fuchsin. (× 1000.)

Among the moulds the varieties most commonly encountered are the *mucor*, the *penicillium*, the *aspergillus* and the *oidium*.

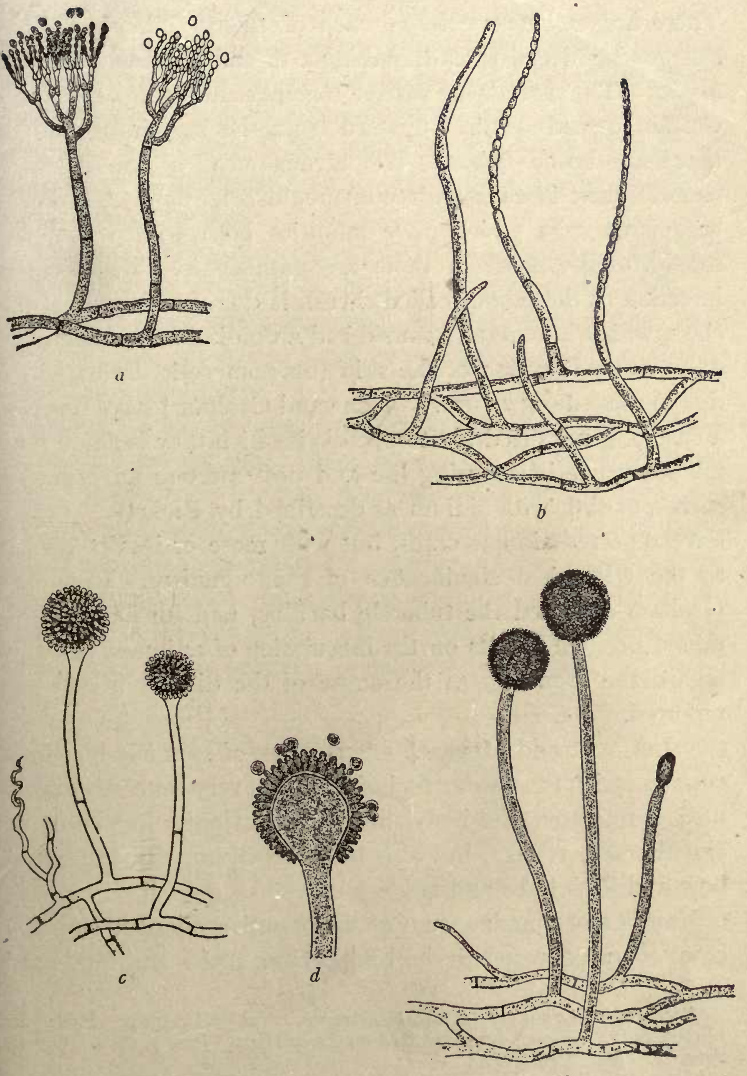

Fig. 63.—(*Baumgarten.*)

a. Penicillium glaucum. *b.* Oïdium lactis. *c.* Aspergillus glaucus. *d.* The same more highly magnified. *e.* Mucor mucedo.

There are various species of each of them. They consist of cells arranged end to end, making a thread-like body called a *hypha*. The threads are matted together and form a *mycelium*. Certain threads project upward from the mycelium, and on them are borne *spores*. The arrangement of the spores is characteristic in each variety of mould (Fig. 63). A group of organisms exist which have affinities both with yeasts and mould-fungi. Some of them are pathogenic. The form of infection of the mouth called thrush is due to a fungus of this class, which is generally considered an oïdium. A chronic inflammatory affection of the skin (blastomycetic drematitis) is due to related organisms.* Irons and Graham† have reported a case of generalized blastomycosis inwhich they isolated from the several lesions during life and post-mortem an organism corresponding with oidium as described by Ricketts. Others have reported similar cases, but with more or less reserve as to the etiological significance of the organism. Irons and Graham excluded the tubercle bacillus, and furthermore obtained positive results on the inoculation of animals, and they regard the organism as the cause of the disease in the case reported.

LeCount and Meyers‡ report a case of systemic blastomycosis in which there were foci of infection very generally distributed throughout the body, involving not only the abdominal and thoracic viscera, but also the cerebellum, and the left elbow and both knee and ankle-joints.

Hamburger§ made a study of the organisms derived from four cases reported respectively by ||Bassoe, Irons and Graham,¶

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*Ricketts. *Journal of Medical Research.* Vol. VI., 1901. Hyde and Montgomery. *Journal American Medical Association.* June 7, 1902. Ophüls. *Journ. Exper. Med.* VI.

†*Jour. Infectious Diseases.* Vol. III., pp. 443–682. 1906.

‡LeCount and Meyers. *Journ. Inf. Dis.* Vol. IV., 1907. pp. 187–200.

§*Journ. Infec. Dis.* Vol. IV., 1907. pp. 201–209.

|| *Journ. Infect. Dis.* 1906. p. 91.

¶ *Loc. cit. supra.*

and Christensen and Hektoen,[*] the last two having reported two of the cases. Hamburger found the four strains to be nearly identical. They grow on all the ordinary culture media, best perhaps on slightly acid glucose media. In cultures from case No. 2 budding forms were not observed. Gross and microscopical differences in the organisms are produced by varying temperatures.

The sporotricha of Schenck[†] which produces chronic subcutaneous abscesses, may be mentioned here, provisionally. A number of skin affections, such as tinea favosa and tinea trichophytina, are due to fungi, which have some similarity to those above mentioned.

Among the mould fungi, several species of aspergillus and of mucor are pathogenic. Man, as well as the lower animals, may be affected. In man the lungs may be involved in a broncho-pneumonia (pneumonomycosis), usually due to aspergillus, and often secondary to some preëxisting disease of the lung. Mould fungi, especially aspergillus, may grow in the external ear (otomycosis). The growth is usually superficial. These fungi rarely produce lesions in other organs.

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[*] *Journ. Am. Med. Assn.* 1906. p. 247.
[†] Hektoen. *Journal Experimental Medicine.* Vol. V.

PART IV.

PATHOGENIC BACTERIA.

Suppuration and Allied Conditions.—The occurrence of suppuration is characterized by certain appearances which we are accustomed to describe under the name of inflammation. The study of inflammation belongs to pathology, and cannot be considered fully here. However, certain evidences which are characteristic of the suppurative variety of inflammation need to be outlined on account of their relation to the action of the pyogenic bacteria.

In a suppurating area, as is well known, the blood-vessels are dilated, and the lymph-spaces become filled with serum. Leukocytes are attracted to the neighborhood in large numbers, by positive chemotaxis, and crowd the small veins and capillaries. The leukocytes, by reason of their ameboid movement, pass through the walls of the vessels at little openings filled with cement-substance, situated between the lining endothelial cells. According to the theory of phagocytosis, they are bent on finding the irritant which has led to the inflammation, and upon attacking it and rendering it harmless. At the point which appears to be the center of the inflammatory area there is usually, but not always, a necrosis of the cells of the tissue; this constitutes the central slough or the familiar core found in some boils. The necrosis is to be attributed to poisons formed by the micrococci. In sections cut through such an abscess the nuclei of the necrotic cells in the center fail to take the nuclear stain; the necrotic mass does not stain, or takes the dye diffusely and irregularly, and it is broken up into fine granules.

The cells of the tissues surrounding the necrotic area are mingled with large numbers of polynuclear leukocytes, which enclose the area of irritation.

The nuclei of the cells near the center of the abscess are frequently broken up into a number of small fragments, which in dicates the commencement of their destruction. In sections through small abscesses it is possible, by means of a double stain of carmine, followed by Gram's method, to bring out the histological character of the tissue, and at the same time to stain the common phyogenic bacteria, which are usually found near the center of the abscess in large numbers, even making masses visible with a low power of the microscope. It is often possible by this method to demonstrate masses of micrococci filling up the lumina of capillaries in which they are lodged as emboli.

The production of pus in the center of the abscess is due to the liquefaction of the necrotic tissue, which apparently results from the action of some peptonizing ferment. In the liquid thus formed immense numbers of the polynuclear leukocytes are found floating, and they constitute the greater part of the so-called *pus-cells.* The nuclei of these cells are obscured by clouds of extremely fine granules. The granules are of an albuminoid nature, and are dissolved by acetic acid, when the nuclei become visible. The nuclei generally consist of three, four, or five more portions. Pus-cells may contain fatty granules; sometimes the cells are necrotic; sometimes living leukocytes may be present. The pus-cells may also contain bacteria. The presence of the fine albuminoid granules in the pus-cells is to be counted as a degenerative change. Although it is possible to produce suppuration experimentally by the introduction of sterilized irritants, such as croton oil, into the tissues of animals, in all cases met with in practice suppuration is due to the action of pyogenic bacteria.

Specimens of pus will nearly always be found to contain bacteria, which can be demonstrated by cultivation, and, as a rule, also in smears made and stained upon cover-glasses. The bacteria in ordinary suppuration lie, for the most part, outside the pus-cells, though some of them may be found in the pus-cells. In the case of the gonococcus and the Diplococcus intracellularis meningitidis they are characteristically found in pairs, inside of, or at least attached to, the pus-cells. The character of the suppuration differs somewhat with the different species of pyogenic bacteria. The kind of abscess above described—localized and having a central slought, usually rather slow in progress—is typical for the Staphylococcus pyogenes aureus, which is prone to produce circumscribed areas of suppuration. The Sterptococcus pyogenes, on the other hand, oftener leads to suppuration of a more diffused character, such as we see in cellulitis and erysipelas; but either organism may, at times, produce the effects usually characteristic of the other. Pus having a blue or green tinge generally owes the color to the presence of the Bacillus pyocyaneus. The commonest pus producing organism is then the Staphylococcus pyogenes aureus, and next to that the Streptococcus pyogenes. Among the other pyogenic bacteria the following may be named:

Staphylococcus pyogenes albus, including Staphylococcus epidermidis albus; streptococcus of erysipelas (probably identical with Streptococcus pyogenes); gonococcus; Diplococcus intracellularis meningitidis; Staphylococcus pyogenes citreus; Micrococcus tetragenus; Micrococcus pyogenes tenuis, which may be the same as the Micrococcus lanceolatus; Staphylococcus cereus albus and flavus.

Pus-formation may also be due to Micrococcus lanceolatus, Bacillus pyocyaneus, Bacillus proteus, Bacillus coli communis, Bacillus pyogenes fœtidus, Bacillus pneumoniæ (of Friedländer), Bacillus aerogenes capsulatus, the ray fungus of actinomycosis, and possibly the bacillus of bubonic plague. Besides these organisms, there are others whose effects are usually more marked in a specific way which sometimes form pus, as the bacilli of diphtheria, tuberculosis, glanders and typhoid fever. Frequently two or more species of pyogenic bacteria will be found associated.

The table below, quoted from Dowd, shows the frequency of the occurrence of various pyogenic bacteria in 135 cases of different types of suppuration.

	Cellulitis, 51 cases.	Infected fresh wounds, 17 cases.	Old granulating wounds, 18 cases.	Healing wounds: stitches, 5 cases.	Furuncles, 7 cases.	Abscesses, 37 cases.
Streptococcus pyogenes alone	9	3	....	....	....	....
Streptococcus pyogenes predominant	23	3	....	....	....	8
Streptococcus pyogenes relatively few	3	1	6	....	....	1
Staphylococcus aureus alone	11	1	1	1	7	6
Staphylococcus pyogenes aureus predominant	8	2	....	....	....	1
Staphylococcus pyogenes aureus relatively few	13	3	....	....	....	2
Staphylococcus pyogenes or epidermidis albus alone	1	4	2	4	....	2
Staphylococcus pyogenes or epidermidis albus predominant	...	1	....	....	....	....
Staphylococcus pyogenes or epidermidis albus relatively few	10	5	3	....	....	6
Staphylococcus cereus albus	3	1	2	....	....	1
Staphylococcus citreus	1	....	2	....	....	1
No growths on agar	....	....	....	....	....	11
Very few growths on agar	....	....	3	....	....	3
Bacillus pyocyaneus	....	....	1	....	....	3
Bacillus coli communis	....	....	....	....	....	3
Overgrown	4	....	2	....	....	1
Few undetermined colonies	12	2	5	....	....	5

The condition of the animal's tissues is of great importance in determining whether or not suppuration will occur when they are exposed to infection. It will be seen that we are repeatedly subjected to infection with pyogenic bacteria, but that in most cases suppuration nevertheless does not occur. The local conditions have an important influence in determining infection. Regions of hyperemia, edema, anemia or necrosis are especially liable to suppuration, as are tissues which have been bruised, lacerated, strangulated or otherwise damaged. Furthermore, the general condition of the patient is of great importance. Chronic diseases and conditions of exhaustion or depression dispose to suppuration, and the depraved condition of the tissues in diabetes renders the sufferer from this disease especially liable to it. These facts have already been enumerated in a previous chapter (page 178). In the lower animals we find that it is often very difficult to produce suppuration artificially with the ordinary pyogenic bacteria. In rabbits the subcutaneous introduction of Staphylococcus pyogenes aureus frequently fails to produce an abscess. Suppuration is likely to result, however, if an irritant body like a piece of sterilized potato or sterilized glass be introduced along with the bacteria.

There are probably a number of other specific predisposing causes in the animal body about which we are only beginning to obtain an understanding. The weakening of the alexins, the absence of opsonins and other intricate conditions are probably subject to great variability, and may serve to explain the tendency to infection at certain times.

Pyogenic bacteria are most frequently introduced into the body through the agency of injuries and wounds of various sorts. They are very widely disseminated in nature and have been found clinging to various objects, especially in city houses. The infection of a wound with pyrogenic cocci, when the suppuration is of a spreading character, such as is most characteristic of streptococcus infection, is known in everyday language as "blood-poisoning." It is possible for infection to take place around hair-follicles through the unbroken skin. In such instances the suppurative inflammation first shows istelf in a minute red pimple with a hair in the center. The pimple presently becomes a pustule. The process may cease at this point, or it may be only the commencement of a large carbuncle with a central slough. Such infection has been produced experimentally on the human skin by rubbing in cultures of Staphylococcus pyogenes aureus. It is, furthermore, the constant experience of post-mortem examiners that infection may occur around the hair-follicles when no wound of the skin has been inflicted.

In many instances, infection with the pyogenic bacteria follows upon some preëxisting infection; this happens, for instance, in tuberculosis, when tuberculous lungs become infected with Streptococcus pyogenes, leading to the formation of a cavity. Secondary infection with pyogenic bacteria is frequently due to the Streptococcus pyogenes, often also to the Micrococcus lanceolatus.

Sometimes it is impossible to detect the point of entrance of pyogenic organisms. In view of the observation that tubercle bacilli pass through the uninjured mucosa without leaving any local lesion, but setting up the disease in places remote from the point of entrance, it may be surmised that the pyogenic organisms may enter the body without leaving any trace of their point of entry.

The severe general symptoms, familiar to every physician, often accompanying acute suppuration, indicate the formation of toxic bacterial products and their absorption. Experimental evidence of the formation of such toxic products is not so clear, however, for the pyogenic organisms as for some of the other bacteria. It has been shown that cultures of Staphylococcus pyogenes aureus, in which the bacteria have been killed, are capable of producing suppuration in the lower animals.

The pyogenic bacteria play a somewhat different part in producing disease, which is fully as important as the typical suppuration seen in an abscess. This happens when the suppurative condition is complicated by other pathological processes, or when there is inflammation of another variety without suppuration. These differences in their action depend largely upon the organ affected. One such condition is osteomyelitis, which is suppuration occurring in bone, but which does not present the ordinary picture of pus-formation owing to the hard and unyielding character of the tissue. Other conditions of very great importance are meningitis, pericarditis, pleuritis, croupous and broncho-pneumonia, peritonitis and endocarditis. It will be observed that these affections are, for the most part, inflammations of the serous membranes. Such inflammations, when they are produced by pyogenic bacteria, are likely to be of great severity, accompanied by the formation of fibrinous exudates; pus-formation may or may not be present. We find that the cause at times is the Staphylococcus pyogenes aureus; this is often the case in malignant endocarditis. Generally speaking, in such inflammations the Streptococcus pyogenes, the Staphylococcus pyogenes aureus and the pneumococcus occur most commonly, although they are by no means the only organisms found. Many cases of peritonitis show the presence of B. coli communis, either in combination with other bacteria or alone.* This is explained by the proximity of the intestine, and especially by the frequent occurrence of peritonitis after perforation of the intestine.†

The process of absorption as it occurs in the peritoneum, as shown by Buxton, and Torrey‡ is as follows:

Solid particles, bacteria or other particles, if injected into the peritoneal cavity are quickly taken up into the lymph channels of the diaphragm thence they are transported through the lymph-glands into the thoracic duct and the blood-vessels. At first the particles lie free in the various organs into which they are carried, but soon they are taken up by phagocytes. The makrophages are the elements mainly concerned in the process. On the injection of typhoid bacilli into the peritoneum of rabbits there occurs an explosive destruction of many of the bacilli, but those which are not so destroyed are taken up as described above.

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* Flexner. Etiology, etc., of Peritonitis. *Philadelphia Medical Journal.* November 12, 1898.

† Buxton and Torrey.

‡ *Journ. Med. Res.* XV., 1906. pp. 1–88. *Ibid.* XVI., 1907.

In inflammations of mucous membranes the common pyogenic organisms are frequently the cause, though other organisms are occasionally responsible. In acute bronchitis, pneumococci and streptococci were found by Ritchie* to be the commonest causes.

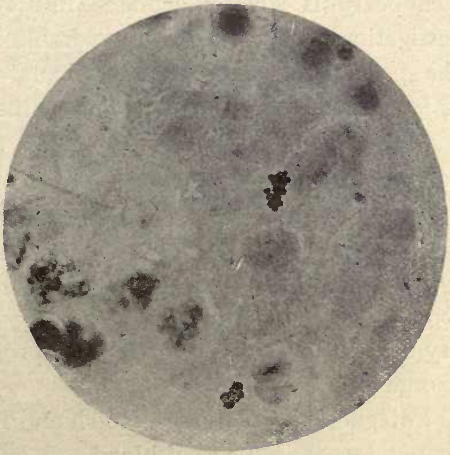

FIG. 64.—Staphylococcus pyogenes aureus in pus stained by Gram's method. (× 1000.)

In inflammations of the middle ear the principal causes are the pneumococcus, the streptococcus and the Staphylococcus aureus and albus.†

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* Ritchie. *Journal Pathology and Bacteriology.* Vol. VII. December, 1900.

† Hasslauer. *Centralblatt für Bakteriologie.* XXXII. Ref. 1902, p. 174. Compare *ibid.*, pp. 240 and 246.

In 25 cases of acute cystitis in women Brown* found B. coli communis, 15 times; S. pyogenes albus, 5 times; S. pyogenes aureus, twice; B. typhosus, once; B. pyocyaneus, once; B. proteus vulgaris, once.

A number of investigators have recovered organisms resembling the pyogenic cocci from cases of acute articular rheumatism. Most frequently a diplococcus or short streptococcus has been found, which has sometimes produced arthritis and endocarditis when inoculated into rabbits. But Cole's† investigations seem rather unfavorable to the view that this is the specific organism for acute articular rheumatism. On the other hand Beattie ‡ comes to the conclusion that the organism is specific for the disease, and that M. rheumaticus, is not an attenuated streptococcus.

From a point where there is suppuration or other localized infection, pyogenic bacteria may enter the circulation and become widely disseminated throughout the body. That happens very commonly in malignant endocarditis. In this manner secondary or metastatic abscesses may be produced in the most diverse organs.

In making a diagnosis in such cases, Rotch and Low§ recommend taking not less than 5 c.c. of blood. A large antitoxin syringe serves the purpose. This is inserted into the most prominent vein at the elbow joint, after scrubbing with soap and water, and washing with ether and alcohol, and a corrosive sublimate pad tied above. Negative results they believe to be of value in prognosis. They believe the method offers valuable means of diagnosis in obscure conditions, cryptogenic septicemia and autointoxication.

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* *Johns Hopkins Hospital Reports.* Vol. X. 1902.

† Cole. Experimental Streptococcus Arthritis in Relation to the Etiology of Acute Articular Rheumatism. *Journal of Infectious Diseases.* Vol. I. No. 4. Nov. 5, 1904. P. 714.

‡ *Journ. Exper. Med.* Vol. IX., 1907. pp. 186–206.

§ *Journ. Am. Med. Assn.* Vol. XLVIII., No. 3. Jan. 19, 1907. pp. 186–189.

The term *pyemia* is used to describe the dissemination of pyogenic bacteria in the circulating blood, with the formation of metastatic abscesses.

Staphylococcus Pyogenes Aureus.—A micrococcus of variable size, arranged in irregular clumps, sometimes in pairs; about 0.8 to 0.9 μ in diameter; not motile (Fig. 65). It stains by Gram's method; it is a facultative anaerobe; grows rapidly, best at 30° to 37° C. It liquefies gelatin. Upon gelatin plates small colonies appear at the end of about two days. It grows well upon all the culture-media. Milk is coagulated. It does not lead to fermentation with the production of gas, but produces various acids.

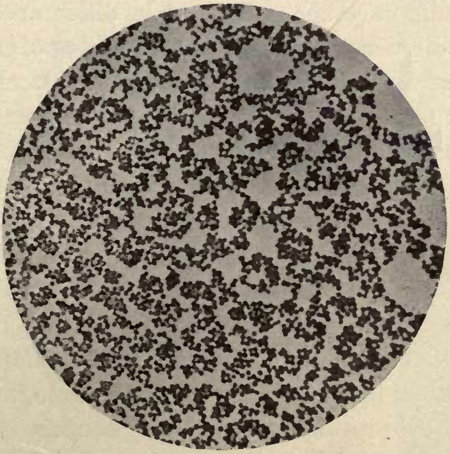

FIG. 65.—Staphylococcus pyogenes aureus, pure culture. (× 1000.)

The growths in the first place are pale, subsequently becoming golden-yellow in color, but only in the presence of oxygen. This color appears well on all media, and is especially distinct on potato. Sometimes the color is slow in developing.

The variability of resistance shown by the organism to higher temperatures was shown by Lübert* was shown to depend upon the manner of application. Suspensions in water were killed in 30 minutes at 50° C. On dried threads it resisted 60° C. for one hour. Aside from the variation shown by different strains of the staphylococcus, the medium in which it is exposed to heat has decided influence upon the effect of the heat, the number of cocci used in the test also has a decided influence. But one-half to one hour at 80° C. appears to kill the organism under all conditions. Cold appears to have no effect. Very variable results have been observed by different authorities on the effect of different chemical agents as well as of electricity and the Röntgen rays. Corrosive sublimate in the strength of 2–1000 requires two or three hours. The many statements in regard to the use of germicides in this connection are confusing. In practice everything contaminated with pus containing the organism should be burned or sterilized in the autoclave for safety. It is not killed by drying alone. In the same specimen the micrococci may have quite different resisting powers to chemical germicides. Some of the individual cells are destroyed by 1–1000 solution of bichloride of mercury in five minutes; others survive exposure to this solution for from ten to thirty minutes.

Sterilized cultures introduced into animals may produce local suppuration. The cells contain intracellular toxic substances.†

As has already been mentioned, the Staphylococcus pyogenes aureus is the commonest of the pyogenic bacteria in man. It has been obtained from a great variety of sources, and appears to be able to exist as a saprophyte. It has been found on the skin, in the mouth, in the nasal and pharyngeal mucus, and also in the alimentary canal. It has furthermore been detected in the air and in dust. It appears to find the conditions necessary for its existence in the vicinity of human habitations.

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*Cited in Kolle and Wassermann. Vol. III., 1903. p. 112.

†Morse. *Journal. Experimental Medicine.* Vol. I., p. 263.

Culture of the Staphylococcus pyogenes aureus vary considerably in virulence. These variations are sometimes to be explained through cultivation on unfavorable media or repeated transplantation from one medium to another; but at times the diminished virulence is due to unknown causes. The lower animals used for experiments are not as readily infected as man. The local introduction in rabbits or guinea-pigs of a part of a culture of Staphylococcus pyogenes aureus may be entirely without effect. The use of a very large dose, or the addition at the same time of some kind of irritant, may produce an abscess. Large amounts of cultures in bouillon may often be injected into the peritoneal cavity of the dog without effect, when the simultaneous addition of a piece of sterile potato or an injury to the gut may lead to fatal peritonitis. Introduction of fluid cultures into the venous circulation of the rabbit generally produces metastatic abscesses in the kidneys, the heart-muscle and the voluntary muscles, and causes death.

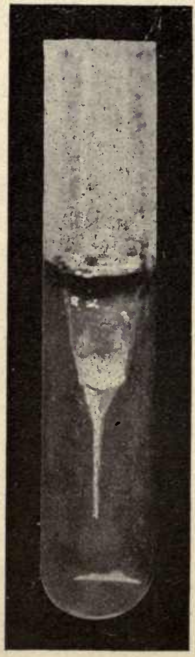

FIG. 66.—Staphylococcus pyogenes aureus. Gelatin culture, one week old.

In man this organism frequently produces suppuration of a merely localized character, such as we are familiar with in boils and carbuncles, but it may also cause generalized infection. It has been shown to be the usual cause of infectious osteomyelitis. Osteomyelitis has been produced experimentally in rabbits by the injection of the Staphylococcus pyogenes aureus, both with and without previous injury to the bone which becomes affected. Ulcerative endocarditis has on numerous occasions been shown to be due to this organism. It has been found possible to produce ulcerative endocarditis experimentally in animals by the injection of the Staphylococcus pyogenes aureus when the valves of the heart have first been mechanically injured. The Staphylococcus pyogenes aureus has also been found in acute abscesses of the lymph-nodes, tonsils, parotid gland and mammary gland, in suppurating joint affections and empyema. It appears, furthermore, in acute inflammation of the serous membranes—pleuritis, pericarditis, peritonitis—although less frequently than the Streptococcus pyogenes.

Staphylococcus Pyogenes Albus.—In form and manner of growth this organism behaves like the Staphylococcus pyogenes aureus, with the exception that it produces no colored growths and its cultures appear white. Its pathogenic properties are less marked, and it is a less frequent cause of suppuration than the Staphylococcus pyogenes aureus. It has, however, been found in acute abscesses on numerous occasions.

Staphylococcus Epidermidis Albus.—According to Welch, the epidermis of man contains with great regularity the organism to which he gave the above name, and which he considers to be a variety of Staphylococcus pyogenes albus. It grows on all ordinary media, it liquefies gelatin, and coagulates milk more slowly than the ordinary Staphylococcus pyogenes albus. It is, furthermore, possessed of less marked pus-producing tendencies. Welch found it impossible to sterilize the skin so as to remove this micrococcus from it. The organism is usually innocuous. It has been found in healthy wounds on numerous occasions. It is capable of causing trouble in wounds when necrotic or strangulated tissues are present, or where a foreign body like a drainage-tube has been left in the wound. It is a common cause of stitch abscesses.

Streptococcus Pyogenes.—Appears as micrococci arranged in chains, usually in pairs, when the adjacent cocci may be flattened. Sometimes the chains are very long. The diameters of the cocci vary from 0.4 to 1 μ. Attempts have been made to create varieties of streptococci according to the length of the chains. On that basis a Streptococcus brevis and a Streptococcus longus have been described.

Buerger* makes a tentative division of streptococci into those which (1) ferment dextorse, levulose, galactose, maltose, saccharose, lactose, inulin, dextrin and mannite; (2) those which ferment all but mannite; (3) those which ferment all but inulin (4) all but inulin and mannite; (5) all but inulin and lactose; (6) all but inulin, mannite, and saccharose.

The Streptococcus pyogenes is not motile. It stains by Gram's method. Escherich† recommended for the staining of the streptococci found in the intestinal canal the following modification of the Gram-Weigert procedure. It is probably available for streptococci in general.

THE STAIN.

A.	Gentian violet,	5 gms.
	Water,	200 c.c.
	Boil a half hour and filter.	
B.	Absolute alcohol,	11 c.c.
	Anilin oil,	3 c.c.

Add 3 parts of A to 1 part of B. This stain keeps for about 2 or 3 weeks.

THE DISCHARGING FLUID.

Iodine-iodide of potassium solution (1 part iodine, 2 parts iodide of potassium, 60 parts water), and analine xylol *mixed in equal* parts. Moreover, pure xylol. For contrast stain saturated alcoholic solution of fuchsin diluted with equal parts of absolute alcohol.

By the method of Hiss (page 49) capsules may sometimes be demonstrated. It is facultatively anaerobic; grows best in the incubator; more slowly at room temperature, and does not liquefy gelatin. In gelatin plates it produces small, round, white, punctiform colonies which are slow of development, and are visible only after about two or three days. It grows on the ordinary media, but according to some authors it does not grow on potato. Milk may or may not be coagulated. The growths are never very luxuriant, and may die out after a few transplantations.

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* *Journal. Exper. Med.* Vol. IX., 1907. pp. 428-435.

† V. Lingelsheim in Kolle and Wassermann. Vol. III., 1903. p. 337.

Streptococcus pyogenes longus is not killed with certainty when suspended in normal salt solution and heated at 60° C. for one hour.* The Streptococcus pyogenes occurs frequently on the mucous surfaces of the healthy body. It is often found in pus, especially pus of spreading inflammations of the kind known as cellulitis. This organism is the commonest infectious agent in puerperal fever, metritis and peritonitis. It occurs commonly in inflammations of the serous membranes—pleuritis, pericarditis and peritonitis. It has been discovered many times in ulcerative endocarditis and in bronchopneumonia. It is frequently present in the false membrane found in genuine

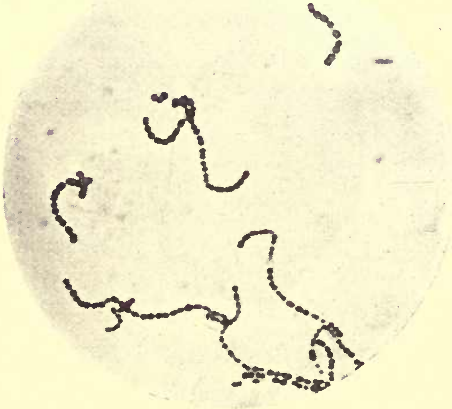

FIG. 67.—Streptococcus pyogenes, from a pure culture. (× 1000.)

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*V. Lingelsheim in Kolle and Wassermann. Bd. III., 1903. p. 318.

diphtheria. It is also the cause of many of the pseudomembranous or so-called "diphtheritic" affections of the throat where the Klebs-Löffler bacillus of diphtheria is wanting. These cases may be indistinguishable clinically from genuine diphtheria, and their nature can be revealed only by bacteriological examination. They are, however, as a rule, milder than genuine diphtheria. The pseudomembranous affections of the throat which occur in scarlet fever and measles are generally caused by the Streptococcus pyogenes, although those diseases may be complicated by genuine diphtheria. Streptococci are very commonly present in the throat in scarlet fever,* and sometimes occur in the blood. Ruediger† using blood agar found streptococcus present in the throat in all the cases of scarlatina—75 cases—which he examined. Hektoen‡ points out that while it is not yet established beyond question

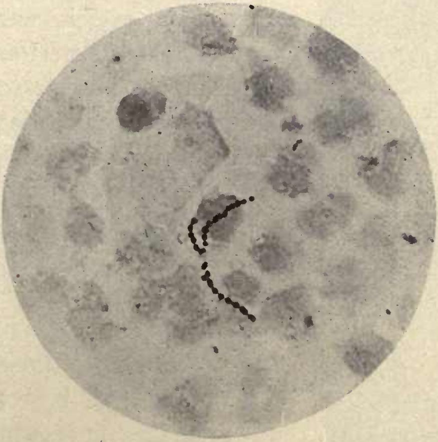

FIG. 68.—Streptococcus pyogenes in pus, Gram's stain. (× 1000.)

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*Weaver. *American Medicine.* April 18, 1903.

†*Journ. Infectious Diseases.* Vol. III., 1906. pp. 755–771.

‡*Journ. Am. Med. Assn.* Vol. XLVIII, No. 14, April 6, 1907. pp. 1158–1160.

that the streptococcus is the cause of scarlet fever, and that it seems more likely that this organism is a secondary invader, nevertheless the Streptococcus pyogenes is always present in large numbers in the throats of affected individuals, and causes many serious complications or it may be a fatal termination. Some observers have produced evidence going to show that scarlet fever is caused by streptococci. Streptococci are very often found in the pustules of small-pox, and may also appear in the blood.

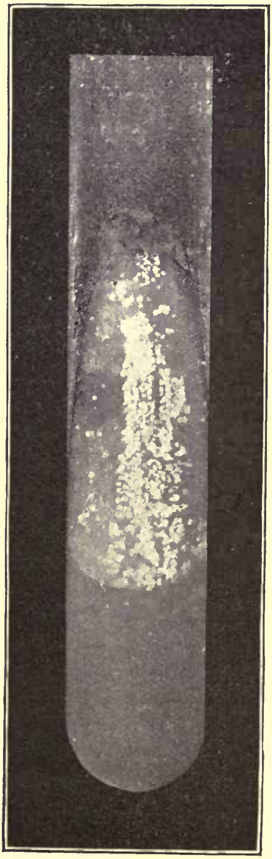

FIG. 69.—Streptococcus pyogenes, culture on agar (slightly enlarged).

The Streptococcus pyogenes is pathogenic for mice and rabbits, but the virulence is very variable. This may sometimes be increased by passing through a number of animals in succession, but is rapidly lost in artificial cultures. It is said that the virulence is best maintained when cultures on gelatin, after forty-eight hours' growth, are kept in a cool place, as in the ice-chest.

A serum of uncertain value derived from an immunized horse or ass has been prepared by Marmorek for the cure of streptococcus infection.

A number of other sera have been prepared for combating streptococcus infection. These have been used in cases of streptococcus infection in human beings; also in cases of scarlet fever.

Van de Velde, and others have prepared a so-called "pollyvalent" streptococcus serum by using streptococci from various sources for the injection of animals. The blood-serum of the injected animals constitute the pollyvalent serum. It is impossible at present to speak definitely in regard to the value of antistreptococcus serum since some seem to have obtained very favorable results with it in practice, while others report no observable improvement in cases treated with it. Since the streptococci belong to that class of bacteria which form endotoxins, the problem of producing an antitoxin is an unsatisfactory matter, to say the least.

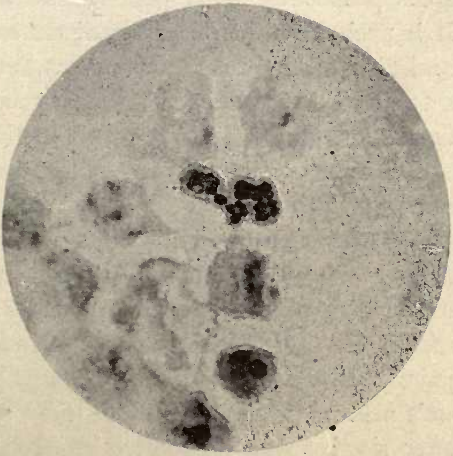

FIG. 70.—Micrococcus tetragenus in pus from a large abscess on the arm, showing capsule. Gram's stain and eosin. (X 1000.)

It is said that streptococci may be agglutinated by serum from animals immunized with streptococcus.

Coley* recommends the use of mixed culture of Streptococcus pyogenes (or S. erysipelatos) and B. prodigiosus in the treatment of inoperable sarcomata. The cultures with which he was very successful in a number of cases were prepared for

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*Reprint from *Med. Record.* July 27, 1907.

him by Buxton. The streptococcus was grown in beef-broth for ten days and the culture then inoculated with B. prodigiosus and the mixed culture grown for another ten days. These cultures, sterilized at 60° C., were injected into the tumors without filtering.

Streptococcus of Erysipelas.—The cause of erysipelas is a streptococcus which in all essential respects—in its morphology, its growth on culture-media, its behavior with stains and its pathogenic properties—corresponds to the Streptococcus pyogenes. It is probable that these organisms are identical, though the clinical manifestations in erysipelas are sufficiently characteristic to justify the clinician in making a distinction between this on the one hand, and the other manifestations of streptococcus infection on the other. In erysipelas, contagiousness is a most marked feature.

Micrococcus Tetragenus.—Found in the cavities in the lungs of pulmonary tuberculosis, in sputum and in pus. The micrococci are enclosed in a transparent capsule, best seen in preparations from the tissues of inoculated animals, and are arranged in pairs or in fours; about 1 μ in diameter; not motile; stain by Gram's method. It grows well at the room temperature, but rather slowly; is a facultative anaerobe; does not liquefy gelatin. Gelatin plates show little, white, punctiform colonies, which, with the low power, are finely granular, and have a peculiar glassy shimmer; in stab-cultures the growths appear as little colonies along the line of puncture. On agar, round white colonies form, which do not tend to spread. It produces a thick, slimy film on potato and a broad, white, moist growth on blood-serum. This organism is only occasionally found in pus. It is pathogenic for white and gray mice and guinea-pigs, but not for rabbits. It may produce septicemia or only a localized suppuration in guinea-pigs. In white mice general septicemia results on inoculation and the Micrococcus tetragenus is found in the blood and in the great viscera. White mice usually die in from two to six days; guinea-pigs in from four to eight days from inoculations.

Micrococcus Lanceolatus (Micrococcus pneumoniæ crouposæ; Micrococcus Pasteuri; Diplococcus pneumoniæ; Micrococcus of Sputum Septicemia; Streptococcus lanceolatus Pasteuri; and Pneumococcus of Fränkel).—This organism was discovered by Sternberg in his saliva in 1880, and afterward demonstrated to be the cause of lobar pneumonia by Fränkel and Weichselbaum. The micrococci usually occur in pairs. The pair of micrococci, in its most typical form, appears like a couple of curved triangles with their bases close to each other. The outline is usually described as being lancet-shaped. The micrococci are frequently oval or round; they often form chains. In preparations made from the blood of infected animals or from pneumonic sputum each pair of micrococci is often seen in stained preparations to be surrounded by a capsule; though even in such preparations the capsule is sometimes difficult to demonstrate; the capsule is not usually seen in preparations made from cultures. For methods of demonstrating the capsule see pages 49 and 121. The penumococcus is not motile. It stains by Gram's method. It grows on potato, but the growth is not visible to the naked eye*

As with most other pathogenic bacteria, the indentification of the pneumococcus is uncertain from observation of its morphology and staining properties alone. These have to be supplemented as in other cases by cultures and animal experiments.

It is facultatively anaerobic. It grows only at elevated temperatures, preferably about 35° to 37° C. Gelatin is not liquefied. It grows well upon agar, upon blood-serum and upon Guarnieri's medium (page 75). Milk usually becomes acid, and may or may not be coagulated. The colonies are seen in their characteristic form upon agar, and are developed after about forty-eight hours, appearing as minute, whitish, translucent, circular growths.

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* Weichselbaum in Kolle and Wassermann. Bd. III., 1903. p. 201.

As a means of differentiation between the pneumococcus on the one hand and the streptococcus on the other, Hiss[*] devised a medium consisting of nutrient agar to which inulin is added. Hiss showed that the pneumococcus ferments this substance in sugar free media, while streptococcus does not ferment it. In fact there is a group of organisms morphologically and culturally like the pneumococcus which do not ferment indulin. Ruediger[†] has made use of Hiss inulin agar for the isolation of the pneumococcus, and he gives the following directions for its preparation and use:

(a)	Peptone (Witte)	10 c.c.
	Agar-agar	15 c.c.
	Sugar-free broth (neutral)	1,000 c.c.

Dissolve by boiling for one hour, adding water from time to time to compensate for evaporation. Heat in the autoclave for 15 or 20 minutes, clarify with egg, filter through cotton and make up to 800 c.c. with distilled water.

(b) Dissolve 15 grams of pure inulin in 200 c.c. of distilled water, mix this solution with (a), add 20 of a 5 per cent. solution of litmus (Merk's highest purity), tube and sterilize in the autoclave under 10 pounds' pressure for 15 minutes. Some stains of pneumococcus will not grow in this medium; it may be improved by adding 1 c.c. of ascetic fluid to each tube before use. In this medium the pneumococcus in 24 to 96 hours at 37° C. show red colonies against a blue background.

Berry[‡] concludes from her investigations that the pneumococcus undergoes decided changes under artificial cultivation, among others in its loss of power of fermenting inulin. These changes may be permanent or they may return under favorable conditions. The change in morphology is seen in a tendency toward a streptococcus type. Loss of virulence is always lost on artificial media, but is enhanced by passage through animals. Berry concludes in regard to the inulin reaction that too much reliance should not be placed upon it, and that a negative result is not sufficient to exclude the pneumococcus, particularly where the organism tested has been cultivated for a long time on artificial culture media.

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[*] *Journ. Experimental Mediciue.* 1905, No. 7, p. 317.
[†] *Journ. Infectious Diseases.* Vol. III., 1906, p. 317.
[‡] Berry. *Journ. Infect. Dis.* Vol IV., 1907, pp. 93–107.

Extensive studies of the pneumococcus were made under the auspices of the Medical Commission for the Investigation of Acute Respiratory Diseases of the Department of Health of the City of New York.* These studies covered the matter of the occurrence of the organisms in the throats of healthy individuals, from persons suffering from various diseases; the various cultural peculiarities, the comparison between the pneumococcus and streptococci, the agglutination reaction, staining peculiarities, and pathogenic properties.

Schottmüller† and Rosenow† independently of one another found that the pneumococcus presented very characteristic appearance when grown on agar to which rabbit or human blood is added. The medium recommended by Rosenow is prepared by adding 0.3 to 0.5 c.c. of sterile defibrinated blood to the tube of melted agar cooled to 45° C. Upon this medium the colonies grow larger than on other media, and they have a distinct green color, and are, furthermore, surrounded by a narrow zone in which the blood-corpuscles are destroyed. This zone is always opaque and has a greenish tinge. The pneumococcus remains viable and retains its virulence for a remarkably long time when it is cultivated upon this medium. Ruediger‡ has shown that the green color of the colonies of pneumococcus in this medium is due to the production of an acid, probably lactic.

Buerger and Ryttenberg§ have come to the following conclusions: The fermentation of inulin is of limited value for the differential diagnosis between pneumococci and streptococci. Pneumococci may lose the power to produce acid in this carbohydrate either temporarily or permanently. Both pneumococci and streptococci may produce precipitation in glucose-serum-agar. Both may cause hemolysis in blood-agar. The diagnosis may be made when everything else fails by use of the capsule staining and animal inoculation. An organism

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*Park and Williams; Collins; Longcope and Fox; Norris and Pappenheimer; Duval and Lewis; Buerger; Hiss and others; Wood; Longcope. *Journ. Exper. Med.* Vol. VII., 1905, pp. 401–626.

†Rosenow. *Journ. Infectious Diseases.* Vol. I., 1904, pp. 280–312.

‡*Journ. Infect. Diseases.* Vol. III., 1907. pp. 663–665.

§*Journ. Infect. Diseases.* Vol. IV., 1907. pp. 609–616.

with pneumococcus capsule staining and streptococcus cultural features may with probability be diagnosed as pneumococcus even though it does not return to the pneumococcus type after animal passage.

It is killed by an exposure to a moist temperature of 52° C. for ten minutes.

It is best cultivated from the blood of an animal which has been infected with the sputum of a case of lobar pneumonia.

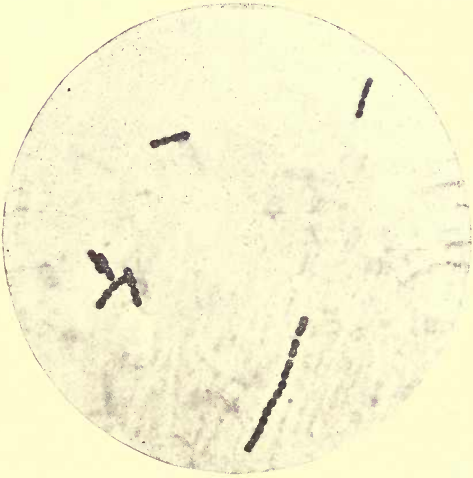

FIG. 71.—Pneumococcus of Fränkel in sputum of pneumonia, Gram's stain and eosin.

Cultures need to be transplanted every few days; they cannot usually be propagated more than a month or two months. The virulence of the organism for animals diminishes rapidly in cultures. It frequently grows as a streptococcus on artificial media. When virulent, it is pathogenic for mice and rabbits; less so for guinea-pigs. In these animals it is likely to lead to fatal septicemia in twenty-four to forty-eight hours when introduced subcutaneously or into the peritoneum or when liquid cultures are injected intravenously. The blood often contains great numbers of the diplococci. The virulence of the organism is very variable. In the sputum of a case of lobar pneumonia, early in the disease, it is likely to be virulent. The virulence is best maintained by repeated inoculations into mice or rabbits.

The Micrococcus lanceolatus has been detected very frequently in the mouths of healthy individuals. But under these conditions it is not, however, pathogenic for animals in many instances, being found virulent in only from 15 to 20 per cent. of such cases. While it is unquestionably the cause of croupous or lobar pneumonia in man in most if not in all cases, there are competent observers who hold that lobar pneumonia is also caused by other bacteria. In that disease the characteristic lesion consists of an inflammation of the lung, involving large areas—usually one or several lobes. An exudate is poured into the air-vesicles, which in the early part of the disease contains red blood-cells, imparting the rusty color to the sputum. The principal element in the exudate is fibrin; it also contains leuko-

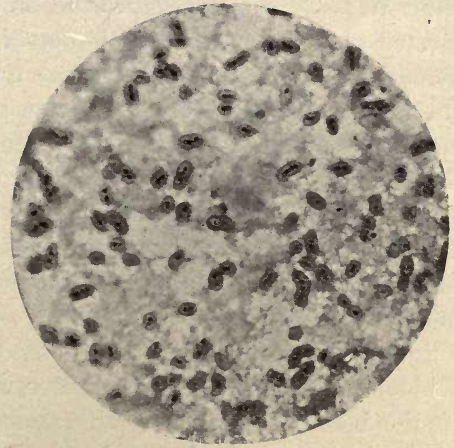

FIG. 72.—Pneumococcus, showing capsule, from pleuritic fluid cf infected rabbit, stained by second method of Hiss.

cytes. The formation of fibrin produces the liver-like consolidation or "hepatization." The diplococci can readily be demonstrated by the Gram method in sections of pneumonic lung, which are best stained by carmine and gentian-violet.

The Micrococcus lanceolatus can be sometimes detected in large numbers, occasionally almost unmixed with other bacteria, in the rusty sputum of lobar pneumonia, often showing the peculiar unstained capsule. On account of its liability to be mixed with other forms of bacteria its presence in the sputum of cases suspected of being pneumonia is not of very great value in differential diagnosis, especially since it is so commonly present in the normal mouth. In a suspicious case its appearance in sputum in nearly pure culture may be significant.

Cultures from the blood of cases of pneumonia, where a large amount of blood is taken, have shown the presence of the pneumococcus in a considerable proportion of the cases, especially when severe or fatal.

Rosenow* found that with proper technic, using large quantities of blood—5 to 7 c.c.—the pneumococcus can be recovered from the blood in practically all cases of croupous pneumonia, and this method may be employed with advantage for diagnosis in obscure cases of the disease. Wolf† found that the pneumococcus is present in the blood in a large percentage of cases even after crisis.

The Micrococcus lanceolatus is often also the cause of bronchopneumonia and of meningitis. Wallstein‡ obtained it in 15 out of 33 cases of primary, and in many cases of secondary bronchopneumonia. It produces inflammations in other situations as well, the most important being pleuritis, pericarditis, endocarditis and arthritis. The Micrococcus lanceolatus may produce pseudomembranous inflammation* and also ordinary suppuration, although not very commonly. It seems also capable of producing ordinary "cold,"† acute catarrh.

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* *Journ. Infectious Diseases.* Vol. I., 1904. pp. 280–312.

† *Journ. Infectious Diseases.* Vol. III., 1906. pp. 446–451.

‡ *Journ. Exper. Med.* Vol. VI., 1905. pp. 391–400.

Pacchioni‡ found the organism in pus from an inflamed knee-joint following, measles with bronchopneumonia and pleurisy as sequellæ. Furrer§ also reports a case of pneumococcus arthritis.

G. and F. Klemperer claim to have obtained toxins from cultures of the pneumococcus, and to have established immunity in animals with the development in the blood of antitoxic substances. Similar attempts have been made by Washbourn and others, but the interpretation of their results at the present time is not clear. The agglutination reaction has been claimed to occur with the pneumococcus, but it does not yet appear to have any practical value in diagnosis.

In regard to the opsonic index (page 216) Wolf‖ has this to say: It first decreases, but rises in favorable cases, and attains its height soon after crisis. In unfavorable cases it remains persistently low.

Organisms related to the pneumococcus have been described under the names of pseudopneumococcus¶ and Streptococcus mucosus.**

The organism named by Rosenbach *Micrococcus pyogenes tenuis* is probably only a variety of the pneumococcus.

Micrococcus Melitensis.—A micrococcus found by Bruce in cases of Malta or Mediterranean fever. It is a round or slightly oval organism, about 0.5 μ in diameter, occurring singly, in pairs or in short chains. It is usually said to be non-motile, though flagella have been described. It is stained by ordinary aniline dyes, but not by Gram's method. It grows slowly, even in the incubator, and more slowly at ordinary temperatures. In gelatin the growth is feeble; there is no liquefaction. On agar pearly white growths appear after three or four days. Bouillon becomes turbid, and later a sediment is formed. On potato there may be slight invisible growth.

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* Cary and Lyon. *American Journal Medical Sciences.* Vol. CXXII.

† Hiss. *Journ. Expr. Med.* Vol. VII., 1905. p. 578.

‡ *Riv. di Clin. Pediatr.* July, 1903.

§ *Arch. for Pediatrics.* July, 1907.

‖ *Journ. Infectious Diseases.* Vol. III., 1906. pp. 731–741.

¶ Richardson. *Journal Boston Society of Medical Sciences.* Vol. V., 1901.

** Howard. *Journal Medical Research.* Vol. VI., 1901. H. S. *Journ. Exper. Medicine.* VII., 1905. p. 547. Buerger *eben.* p. 497.

A commission sent by the Royal Society of England * came to the conclusion that Malta fever is spread through goats' milk. The report of this, as it appears in the Journal quoted, does not seem convincing.

Malta fever occurs chiefly about the Mediterranean. It has been observed in India, in the Philippine Islands and in Porto Rico.

It is a chronic febrile disease, but not very fatal, accompanied by pains in the joints and perspiration. At autopsies the organisms may best be recovered from the enlarged spleen. Accidental infection, sometimes fatal in man, has occurred from pure cultures on a number of occasions. The disease may be reproduced in monkeys by inoculation with pure cultures. The agglutination reaction is positive in this disease. The diagnosis is best made by applying this test to the blood-serum of the patient, with a known pure culture of Micrococcus melitensis.† For this purpose a suspension of an agar culture is made in normal salt solution. The diluted serum is added so as to secure a dilution of about 1–50, but the dilutions used have varied widely. Precipitation quickly follows agglutination. According to Craig, the test may be made on a slide, examining with the microscope as for the typhoid bacillus (see Serum-test for Typhoid Fever).

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* London letter to *Journ. Am. Med. Assn.* June 1, 1907.

† Musser and Sailer. *Philadelphia Medical Journal.* December 31, 1898, July 8, 1899. Strong and Musgrove. *Ibid.* November 24, 1900. Curry. *Journal Medical Research.* Vol. VI. 1901.

Diplococcus Intracellularis Meningitidis.*—Found in the exudate of cerebro-spinal meningitis by Weichselbaum; isolated by Dunham† and others from the upper air passages in such cases.

Flexner‡ found that the reason for the rapid death and disintegration of the organism which takes place in 24 to 48 hours in the ice box and less quickly in the incubator is due to an enzyme contained in the organism itself—an autolytic enzyme.

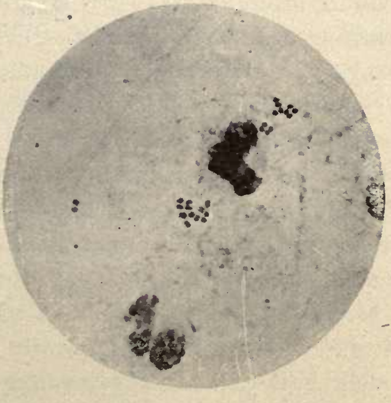

FIG. 73.—Diplococcus intracellularis meningitidis and pus-cells. (× 1000.)

Flexner furthermore found that by using diluted serum of the sheep instead of that of human beings in the agar medium, and by the addition of a calcium salt (calcium carbonate) to this sheep-serum-agar medium a given culture will remain alive many weeks.

Dunham and Ward, assisting Flexner, failed to find the organism in the throats and nasal cavities of a number of domestic pets and of persons not suffering from meningitis.

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* The writer is indebted for the brief statement which it is possible to give here chiefly to the exhaustive Report to the Massachusetts Board of Health by Councilman, Mallory and Wright, 1898. The photograph was made from a preparation kindly furnished by Dr. Mallory. See also Davis *Journ. Infec. Dis.* Vol. IV. 1907. pp. 558–681.

† *Journ. Infect. Dis.* Sup. No. 2. Feb., 1906. pp. 10–20.

‡ *Journ. Exper. Med.* Vol. IX. 1907. pp. 105–141.

A micrococcus about the size of the common pyogenic cocci; grows in fours, but more often in pairs consisting of two hemispheres separated by an interval which does not stain; usually found within the pus-cells, in which respect it resembles the gonococcus. It is stained by ordinary methods with the aniline dyes, and is decolorized by Gram's method. It does not grow at the room temperature, but only in the incubator; gelatin is not available as a culture-medium. There is no growth on potato and scanty growth on agar or in bouillon. The development is most abundant upon Löffler's blood-serum, when round, white, shining, viscid-looking colonies with sharp outlines may be seen in twenty-four hours. The serum is not liquefied. Upon agar, or better upon glycerin-agar, the colonies are flat, round, translucent, viscid-looking, having a yellowish-brown color under the low power. The organism should be transplanted to fresh media frequently, as it rapidly loses its power of reproduction. Many of the tubes inoculated with the original material or with pure cultures show no growth.

It is moderately pathogenic for guinea-pigs and rabbits when inoculated into the pleura or peritoneum. Meningitis and encephalitis have been produced in the dog and goat by inoculation in the meninges.

Flexner (*loc. cit.* pp. 141–167) found that injection of the organisms into the spinal canal of monkeys produces a disease the symptoms and lesions of which bear close resemblance to those occurring in the natural infection in man. Injection of monkeys into other parts of the body of monkeys produce only local effects.

This organism appears to be the principal if not the only cause of epidemic cerebro-spinal meningitis. The lesion consists of a purulent inflammation of the pia and arachnoid, extending into the brain-substance, over the cord and along the nerves. General invasion of the tissues of the body seems not to occur, but focal areas of pneumonia may be present. Spinal puncture in the lumbar region is recommended as a means of diagnosis. The fluid should be examined with the microscope and by cultures.

Flexner (*loc. cit.* pp. 168–185) has made use of antisera, which counteract the fatal effects of injections of intracellularis. He also found that this could be effected to a less extent by normal sera and other fluids. The trials of antiserum obtained from the horse have been tried in a number of cases upon human beings with apparently favorable results, and Flexner and Jobling* state that while they are not finally convinced of its value, they believe the data so far warrants a wider trial. The organism was obtained by Warfield and Walker† from a case of endocarditis with general septicemia.

Micrococcus Gonorrhœæ (Gonococcus of Neisser).—Found in pus in cases of gonorrhea. The micrococci generally are in pairs, occasionally in groups of four. The cocci are flattened, the flattened sides facing each other, and they are often compared to a pair of biscuits. The long diameter of the pair of biscuit-shaped elements is about 1.25 μ. The organisms are usually found attached to the epithelial cells or inside of the pus-cells; they are also found in smaller numbers floating free in the fluid. They stain with ordinary aniline dyes, for example, Löffler's methylene-blue, but not by Gram's method.

The fact (1) that the cocci, after the acute stage at least, in carefully prepared specimens are always found largely inside of the pus-cells, (2) that they are in pairs of biscuit-shaped micrococci, (3) that they are not stained by Gram's method, will serve to distinguish the gonococcus from all the other ordinary pus-forming bacteria and from the meningococcus and M. catarrhalis. There are other diplococci (pseudogonococci),

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**Journ. Exper. Med.* Vol. X., 1908. p. 202.

†Bull. Ayer Lab., Univer. Penna. Hosp. No. 1, Oct., 1903.

probably non-pathogenic, which have been found occasionally in the vulvo-vaginal tract and in the uretha, which, it is said, are also decolorized by Gram's method. Such organisms are not likely to present all the points mentioned as characteristic of the gonococcus. The recognition of the gonococcus in the discharges of a case of acute gonorrhea is usually an easy matter. It must be admitted, however, that in cases having chronic discharges, when its detection is most to be desired, the diagnosis may become very difficult and is frequently impossible, except by culture-methods, owing to secondary infection with the ordinary pus-forming or other bacteria, which may be present in larger numbers than the gonococci themselves.

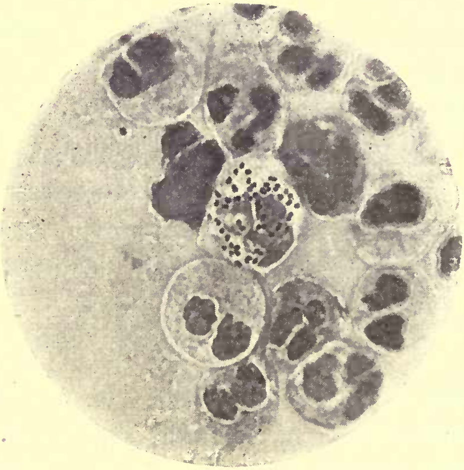

FIG. 74.—Gonococci and pus-cells. (×1000.)

The gonococcus grows only in the incubator, and cannot therefore be cultivated upon gelatin. Its cultivation is, in fact, a matter of some difficulty. The medium usually selected is a mixture of agar with human blood-serum. The blood-serum from the placental blood or pleuritic or peritoneal transudates, or hydrocele fluid, has been employed. Human urine, sterilized by filteration through porcelain, added to the mixture of blood-serum and agar, improves its character, according to some writers.

Baer* recommends the following medium: Hydrocele, pleuritic, or ascitic fluid should be caught under aseptic conditions in sterile flasks, distributed into test-tubes, and tested for sterility in the incubator for 24 hours at 37° C. This is mixed with plain agar which has been previously condensed to two-thirds its bulk in the proportion of two parts agar to one of the transudate. The transudate is added to the agar in tubes, the agar having been melted and cooled to 45° C. The tubes so prepared are allowed to solidify in a slanting position, capped with rubber caps which have been sterilized in a 1–1000 corrosive sublimate solution, and then placed in the incubator again for two days to test their sterility. The slant tubes may be used for plating out the gonorrheal pus by taking a loopful of the material to be examined and smearing it over the surface of the agar, using the condensation water in the tube to assist in the spreading. Other organisms identical in morphology and in staining properties, including the negative Gram reaction may be cultivated from suspected gonorrheal discharge, but these grow on ordinary culture media. A poison has been extracted from cultures of the gonococcus which produces toxic symptoms in various animals.†

The colonies of the gonococcus are very small, grayish-white, circular, translucent; appearing after from twenty-four to forty-eight hours. They may attain a diameter of 1 to 2 mm. The gonococcus will occasionally develop on ordinary glycerin-agar or Löffler's blood-serum medium, but the growth is likely to be feeble and cannot be relied on. The cultures live for a

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Journ. Infect. Dis. Vol. IV., 1904. pp. 313–326.

†Neisser and Scholtz in Kolle and Wassermann. Bd. III., 1903. p. 174.

considerable time if kept from drying. The gonococcus is not known to produce urethritis or conjunctivitis in any of the lower animals. In the peritoneum it may cause suppurative inflammation in mice and guinea-pigs. Reproduction of the disease in man has been effected by experimental inoculation with pure cultures. Besides being the cause of gonorrheal urethritis and infection of the cervix uteri, the gonococcus has been isolated from cases of vaginitis in little girls and from gonorrheal conjunctivitis. It has been found to be the cause of many cases of pyosalpinx, as well as of gonorrheal proctitis, naphritis, arthritis, myocarditis and endocarditis; these conditions complicating gonorrhea may also be secondary or mixed infections.

Bacillus of Soft Chancre (of Ducrey).—A small, oval bacillus, usually occurring in chains. It stains with ordinary aniline dyes, but not by Gram's method. It has been cultivated on human blood-agar (also rabbit blood-agar; the medium deteriorates in a few weeks—Davis). It is cultivated with difficulty. It is found in the pus of soft chancre or chancroid, usually mixed with other organisms. It has been demonstrated in sections of the ulcers. There seems to be uncertainty with respect to its occurrence in buboes. Ducrey was able to secure it in pure culture by successive inoculations on the human skin. Although this bacillus has not yet been sufficiently studied, there seems little doubt that it is the cause of soft chancre.*

Bacillus Pneumoniæ (of Friedländer), or *Bacillus mucosus capsulatus*.†—A short bacillus with rounded ends, sometimes growing out to a greater length; sometimes occurring in pairs; surrounded by a capsule which is seen only in preparations made from the tissues of infected animals, and is not seen in cultures. This bacillus is not motile. It does not form spores.

*Davis. *Journ. Medical Research.* Vol. IX. 1903.

†Howard. *Philadelphia Medical Journal.* February 19, 1908. Curry Howard, Perkins. *Journal Experimental Medicine.* Vols. IV. and V.

It stains with the ordinary aniline dyes, but does not stain by Gram's method. It is aerobic and facultatively anaerobic. It may be cultivated at ordinary temperatures, but grows best at high temperatures. It does not liquefy gelatin. Stick-cultures in gelatin develop a round, flat knob at the point where the puncture enters the surface of the gelatin, making what is called a "nail-shaped" growth; the growth in gelatin is white; in old cultures the various media acquire a brown color. Dextrose and lactose are fermented by it; in cultures on potato, gas is formed, causing a frothy appearance; milk is not coagulated. It does not produce indol.

The thermal death-point is about 56° C. moist heat. Strains of this organism are pathogenic for mice, less so for guinea-pigs and rabbits. This bacillus is sometimes found in the healthy mouth and nose. It has been known to cause inflammation, especially in the eyes, mouth, nose and ear; also bronchopneumonia, and more rarely empyema and meningitis. It was described by Friedländer as the specific cause of lobar pneumonia; but more recent investigations indicate that it is comparatively seldom found in this disease.

There are various capsulated bacilli (capsule bacilli of R. Pfeiffer and others) which closely resemble the bacillus of Friedländer, and at least belong to the same group. The bacillus of ozena, which has often been found in that disease is very similar. B. lactis aerogenes and B. coli communis also have many points in common with the Friedländer bacillus.

Perkins,* as a result of his studies of this organism, comes to the conclusion that there is no one organism entitled to the name exclusively, but that the term includes a large number of organisms which have been given various names. The only method of differentiating the different members of the group is by noting the fermentation reactions with sugars. He makes three tentative groups. All carbohydrates fermented with the formation of gas; all carbohydrates except saccharose fermented with the formation of gas: all carbohydrates except saccharose fermented with the formation of gas. He would call the first group *Bacterium aerogenes;* the second, *Bacterium pneumonicum*, the third, *Bacterium acidi lactici.*

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* *Journ. Infec. Dis.* Vol. I. 1904. pp. 241–267.

Under Bacterium aerogenes he would include *Bacterium aerogenes* already so called, *B. capsulatus septicus*, and several cultures with various names obtained from different sources: Several from Johns Hopkins University labeled "*B. pneum. Friedländer*," *B. hemorrhagic septicemia Howard*, *B. mucosus Blumer*, *B. mucosus capsulatus Wright and Mallory*, besides others.

Under *Bacterium pneumonicum Friedländer*, *B. capsulatus Fashing*, *B. sputiginus crassus*, *B. ozenæ*, and probably *B. rhinoscleromæ*, and other of the Johns Hopkins cultures.

Under *Bacterium acidi lactici* he would include the organism going by this name.

Infections due to this organism are very prevalent in Perkins locality (Cleveland, O.), and are caused almost exclusively by members of the *Bact. aerogenes* division.

Bacillus of Rhinoscleroma.*—A short bacillus with rounded ends, often united in pairs, also growing to a greater length; surrounded by a capsule; not motile; stained by the ordinary aniline dyes. It is much like the bacillus of Friedländer, but some writers state that it retains Gram's stain more tenaciously than that organism; this may be doubted, however. The organism has been cultivated. It is a facultative anaerobe. It grows rapidly, best in the incubator. It does not liquefy gelatine; its growth in gelatin stick-cultures, resembles that of the bacillus of Friedländer. It grows on the ordinary media. Gas may be developed upon potato.

It is pathogenic for mice and guinea-pigs, less so for rabbits. Its virulence is less than that of Friedländer's bacillus.

It has been obtained from the tissues of cases of rhinoscleroma. Rhinoscleroma is a disease characterized by a chronic tubercular thickening and swelling of the skin around the nose and similar swelling of the nasal mucous

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*Perkins comes to the conclusion from his investigations that this organism has no etiological connection with the disease in question, but that it is rather a secondary invader. *Journ. Inf. Dis.* Vol. IV., No. 1. p. 65.

membrane, sometimes followed by ulceration. It is commonest in Austria and Italy. It has been seen in America only very rarely.

The organisms may be stained in the diseased tissues, but their detection is a matter of considerable difficulty, and they are not always found. It is not yet certain that they are the cause of rhinoscleroma.

Bacillus Mycogenes.*—A plump, short bacillus, less than 1 μ in breadth, possessing no flagella, non-motile, does not form spoes; capsules are seen in preparations from tissues of inoculated animals and in milk cultures, rarely in preparations from agar cultures. The organism occurs singly or in pairs, and even in longer filaments. Gram positive in tissues, but negative in cultures.

The growth on agar is porcelain white and viscid. In all liquid media viscidity is very marked. Gelatin is not liquefied. Coagulated blood-serum not liquefied. "Nail-head" growth shows in stab cultures. Milk is coagulated in one to five days. Casein not digested. Litmus is reduced. Growth on potato is brown and slimy, but there is no gas formation. Indol negative. None of the sugars are fermented.

Very pathogenic for rabbits and guinea-pigs. Rabbits are killed in eighteen hours by subcutaneous injection of $\frac{1}{100}$ c.c. of a twenty-four hour beef-broth culture, guinea-pigs in less than fifteen hours by the same dose.

Bacillus Pyocyaneus.—A slim bacillus with rounded ends. It is motile. It does not form spores. It is decolorized by Gram's method. It is aerobic; grows well at ordinary temperatures; liquefies gelatin, and grows on the ordinary culture-media. Cultures persent a blue or green color, especially in transparent media. This color is not confined to the growth itself, but a blue or green fluorescence spreads over the whole medium. In an old agar culture the color may become very dark. The pigment forms in the presence of oxygen, and is due, at least in part, to the ptomaine, pyocyanin. On potato

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*Ralph T. Edwards. *Journal of Infectious Diseases.* Vol. II., No. 3. 1905.

the growth is usually brown; the surrounding medium may be tinged with green. Milk is coagulated and peptonized and an acid reaction is developed. Indol is formed in Dunham's peptone solution. Coagulated blood-serum is liquefied.

The Bacillus pyocyaneus seems to be rather widely distributed in nature; it has been found on the skin, in normal feces, also in diarrheal discharges and in dysentery. It is the cause of the color in blue or green pus. It has frequently been demonstrated in pus, but oftenest perhaps, in mixed infections. It has been found in various abscesses, in otitis media, peritonitis, appendicitis and bronchopneumonia. It has been known to produce general septicemia.* It is pathogenic for guinea-pigs and rabbits, in whom it may produce septicemia. In animals it may lead only to local suppuration, from which they

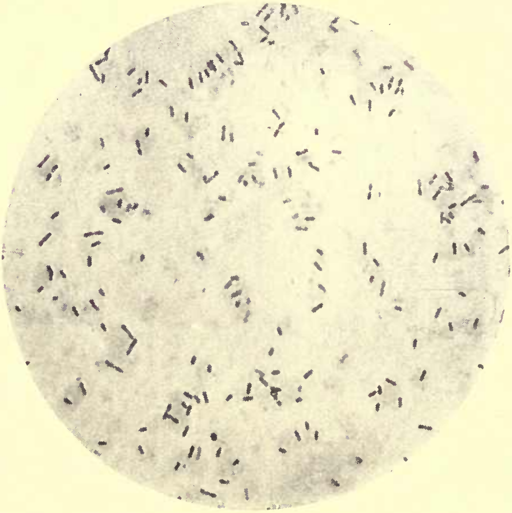

FIG. 75.—Bacillus pyocyaneus pure culture. (× 1000.)

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*Lartigau. *Philadelphia Medical Journal.* September 17, 1898. *Journal Experimental Medicine.* Vol. III. 1898. Perkins. *Journal of Medical Research.* Vol. VI. 1901.

may recover, being made immune from subsequent infection with this organism.

Emmerich* has obtained a bacteriolytic enzyme by passing cultures of B. pyocyaneus three weeks old through Berkefeld filters. To this substance he has given the name "pyocyanase," and he finds that it not only disintegrates the bacillus pyocyaneus itself, but also the bacteria of cholera, diphtheria, typhoid, plague, anthrax, also streptococci, staphylococci, and gonococci. Tubercle bacilli and the hay bacilli are not affected by the enzyme. The substance has been used with very favorable results not only in experiment upon animals, but also in human diphtheria, not only in dissolving the membrane in the throat in these cases but in neuralizing the toxin of the disease. Reports on all sides seem so far very favorable to its use as a therapeutic agent.

There appears to be a whole group of fluorescent organisms of slightly different characters which closely resemble one another, all classed as pyocyaneus.

Bacillus Proteus.—A bacillus with rounded ends, varying much in length, breadth 0.4 to 0.6 μ; frequently appearing as short ovals like micrococci; sometimes growing out into long filaments, so that it is said to be pleomorphic. Rounded involution forms occur. It is not stained by Gram's method. It is motile. Spore formation has not been observed. It is aerobic and facultatively anaerobic. It grows rapidly at ordinary temperatures. This organism was originally described by Hauser as three different species—*Proteus vulgaris*, which was said to liquefy gelatin rapidly, *Proteus mirabilis*, which liquefied gelatin slowly, and *Proteus Zenkeri*, which did not liquefy gelatin. It seems probable that these organisms were, in fact, varieties of the same species, now called Bacillus proteus. To proteus vulgaris or some closely allied form has

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***Münchner Med. Wochenschr.* Nov. 5, 1907. Vol. LIV., No. 45. Cited in *Journ. Am. Med. Assn.* Dec. 14, 1907.

been attributed the causation of several cases of poisoning from spoiled meat.* Upon gelatin-plates the colonies present a characteristic phenomenon, when seen under the low power, in the projection of processes which subsequently change their form and position, and which may become entirely detached from the original colony, so that the surface of the gelatin may become covered with so-called "swarming islands."

The proteus grows on the usual media, tending to produce a foul odor, decomposition and alkaline reaction. In urine it converts urea into ammonium carbonate.

This organism is one of those which were formerly described under the name of Bacterium termo. It is among the most common and widely distributed bacteria. It has been found in decomposing animal and vegetable substances, in the feces, in the urine in cystitis and in the discharges of children suffering from cholera infantum. It appears that this organism may occasionally be pathogenic to man, causing pus formation, peritonitis and even general infection.† Cultures injected in considerable amounts may be pathogenic to animals.

Bacillus of Bubonic Plague. (Bacillus *sive* Bacterium Pestis Bubonicæ).—An oval or short rod-shaped bacillus, with rounded ends, sometimes possessing a capsule. It occurs singly or in pairs rarely in chains. Involution forms are met with in material from old buboes. Branching forms have been noted.‡ It is not motile. It does not form spores. With the aniline dyes the ends stain more deeply than the middle; this is called polar staining; by Gram's method it is decolorized. It is aërobic. It grows at ordinary temperatures, but better in the incubator. It grows on most media. The growths are grayish-white. Gelatin and blood-serum are not liquefied. In bouillon, the medium remains clear, while a granular deposit forms on the sides and

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*Günther *Loc. cit.* p. 709.

†Ware. *Annals of Surgery.* Vol. XXXVI. 1902.

‡Kolle. *Zeitschr. f. Hygiene.* Bd. 36. 1901. p. 399.

bottom of the tube. In bouillon to which a few minute drops of sterile oil, as cocoanut oil, have been added, a growth takes place from the under side of the oil drops. Such growths extend down, and are called stalactite growths. The stalactites break off, with the slightest disturbance.

Remarkable involution forms appear on agar containing 3 per cent. of common salt. The stalactite growths and the forms occurring on salt-agar are considered the most characteristic cultural peculiarities.*

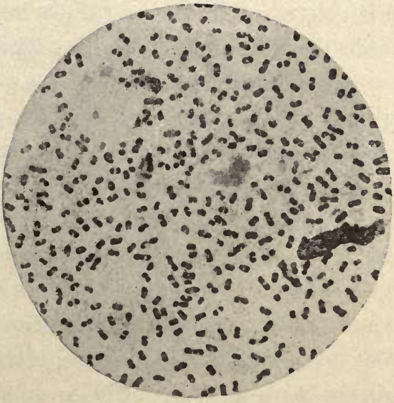

FIG. 76.—Bacillus of bubonic plague.—(*Yersin.*)

It is sometimes sensitive to drying, but may sometimes survive prolonged drying. When spread in thin layers, it is killed in three to four hours by direct sunlight. Dried out on cotton and linen cloth the bacilli were found to be still alive after 18 hours exposure to sunlight. The action of sunlight is in direct proportion to the thinness of the layer into which the cultures are spread, being seriously hindered when the layer is thick, in a few minutes by steam at 100° C. Rosenau states that it is killed in one hour by one per cent. carbolic acid, but others

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*Wilson. *Journal Medical Research.* Vol. VI. 1901.

state that it requires longer than this. Kolle and Wassermann quote various authorities, none seem to agree. Abel states that it required two hours in this strength. Gioxa and Gosio, three hours. In solutions of higher strength the discrepancy between authorities varies also; in 5 per cent. solution from one to ten minutes, one hour in 1 per cent. carbolic acid.* It is pathogenic for rats, mice, guinea-pigs, rabbits and a number of other animals besides flies and other insects. The rat-flea, pulex cheapis, has been shown by the British Commission† to bite human beings; the inference from this is that the plague bacillus is conveyed from the rat to man in this way. Thompson‡ holds that the flea is the intermediary between the plague rat and human beings.

In man it appears usually to enter through wounds of the skin. Other possible avenues of infection are the air-passages, the mouth and the gastro-intestinal tract. In plague three different clinical forms are to be recognized—the bubonic, the pneumonic and the septicemic. The *bubonic* form is commonest. The point in the skin at which the inoculation takes place seems generally to exhibit no inflammatory reaction. The lymph-nodes are generally swollen, especially the deep inguinal and axillary nodes. The swollen lymph-nodes may suppurate. The suppurating nodes are often infected simultaneously with micrococci. The bacilli are numerous in the enlarged lymph-nodes, but may be detected in the other organs of the body and in the blood. The organism is furthermore to be found in the fluid aspirated from buboes during life. It may be cultivated from this fluid, and recovered from rats and guinea-pigs inoculated with it. In the *pneumonic* or pulmonary form the bacilli occur in the sputum, and may be tested in the same manner. This type of the disease is said to be very fatal. In the *septicemic* form no primary bubo is found; but a bubonic case may become septicemic, and this form is also very fatal.

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*Rosenau. Viability of Bacillus pestis. Marine Hospital Service. Hygienic Laboratory Bulletin. No. 4. 1901.

†London letter to *Journ. Am. Med. Assn.* Nov. 16, 1907. See also *Ibid.* Vol. L., No. 2. Jan. 11, 1908. p. 127.

‡*Australian Medical Gazette.* Nov. 20, 1907. Abs. *Journ. Am. Med. Assn.* Jan. 18, 1908. p. 127.

During epidemics of plague it has been noted that rats may die in large numbers, and plague bacilli have often been recovered from the bodies of such rats. The systematic destruction by health departments of all the rats possible is important where an epidemic is present or is feared. The same applies to mice. The agency of fleas as carriers of the bacilli has been suggested, and according to Thompson* this has been proven. Flies have also been suggested as carriers.

No one but the most experienced and strictly careful person should trust himself with experiments with cultures of the plague bacillus. The danger is so great, not only to the worker himself, but to those around him that the cultures should be handled in a room where only the worker himself is engaged in his experiments. It follows, of course, that cultures should under no circumstances be entrusted to classes of students under instruction.

The greatest care must be used in working with the bacillus of plague. A number of fatal results have occurred through it in laboratory investigators.

Haffkine has devised a method of protective inoculation against plague consisting of the injection of small doses from cultures in which the bacilli have been killed. An accidental infection with tetanus at the time of injection in 19 persons in India rather discredited this method for a while, but subsequent results have been very encouraging. An active immunity which is quite lasting, it is maintained, may be secured by this method in some days. The injection is sometimes

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*J. A. Thompson. *Australian Medical Gazette.* Sidney, Australia. Nov. 20, 1908.

followed by considerable constitutional disturbance. This method seems likely to be of considerable value.

Yersin and others have prepared protective sera on the same general principles used in making other sera for effecting passive immunity. The results so far obtained with these sera are not especially encouraging.*

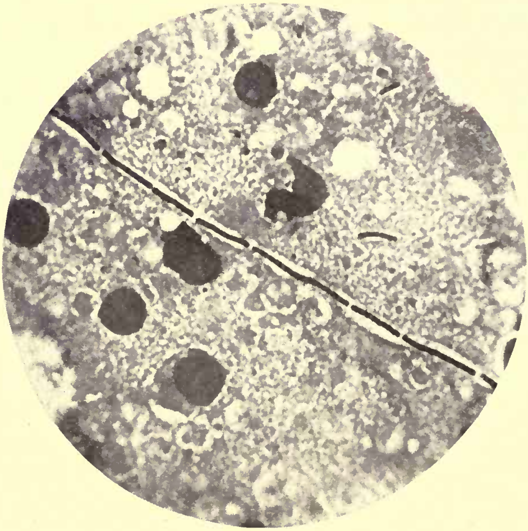

FIG. 77.—Bacillus aërogenes capsulatus, smear preparation from rabbit's liver.. (× 1000.)

An agglutination reaction has been described; but this is not likely to be of great value in diagnosis.

The period of incubation in this disease is from two to seven days. It has occasionally appeared in civilized countries during recent times, though not to a very serious extent. Among the localities of importance to us it has recently visited the Philippine Islands, California and Mexico. It has ravaged

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*Günther. *Loc. cit.* p. 630.

the southeastern part of Asia within a few years. In the Middle Ages, and in succeeding centuries, it devastated many of the countries of Europe, where it was one of the most important of the pestilences that went in those days by the name of the "plague." It appears to have been the disease known in English history as the "black death."*

Paraplague Bacillus.—Neumann† described an organism which he isolated from rats in Hamburg which differs from B. pestis bubonicæ only in that it produces no results when injected subcutaneously or intraperitoneally into rats and, furthermore, in that agglutination tests were negative. Breathing into the lungs of very minute quantities was fatal for rats.

Bacillus Aerogenes Capsulatus.—A thick bacillus, 3 to 6 μ in length, frequently capsulated, discovered by Welch and Nuttall. The capsules may be found in preparations from animal tissues, but rarely in cultures. It sometimes forms spores, chiefly in cultures on blood-serum. The vegetative forms are destroyed at 58° C. moist heat in ten minutes, but the spores withstand boiling nearly eight minutes. It is not motile. It stains by Gram's method. It is anaerobic, and is readily cultivated by Buchner's method for anaerobes. It grows best at the body temperature, but will grow at the room temperature. It may liquefy gelatin slowly or not at all. The growths are whitish. In media containing lactose, dextrose or saccharose it produces an abundance of gas; but according to Welch, it is also able to form gas from proteids. Milk is coagulated, and the reaction becomes acid. Gas forms upon potato, where the growth is thin and grayish-white.

It occurs in the intestine of man and various other animals, in soil, sewage and water. It is not usually pathogenic for rabbits and mice. In guinea-pigs, sparrows and pigeons it may

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*For further details concerning plague consult articles by Parker, Novy and Flexner. *Transactions of the Association American Physicians.* 1902. Calvert. *American Medicine.* January 24, 1903.

†*Zeitschr. f. Hygiene.* XLV., No. 5. 1903.

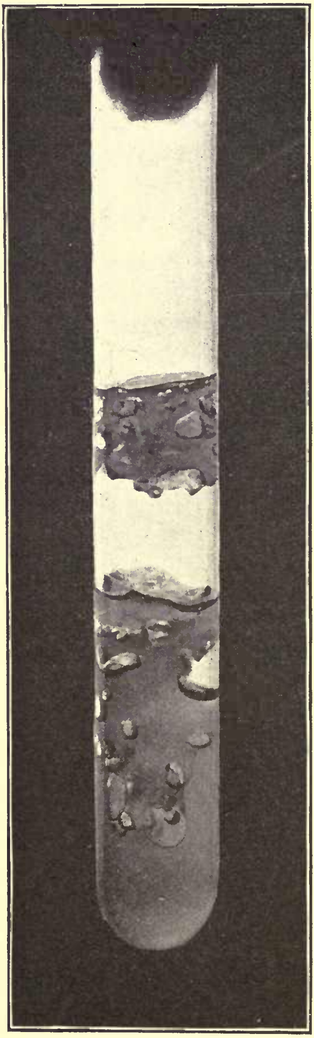

FIG. 78.—Bacillus aërogenes capsulatus, culture in dextrose-agar, showing gas-bubbles.

produce "gas phlegmons." It has been found on numerous occasions in the organs of human cadavers in which a development of gas had taken place, producing bubbles or cavities in the tissues, imparting to them a peculiar spongy character (German, *Schaumorgane*). Probably this is, as a rule, a postmortem invasion, but there is reason to believe that in some cases it enters the circulation during life. It has been found in cases of emphysematous gangrene or cellulitis, in various uterine infections, including physometra and emphysema of the uterine wall, in pneumothorax and pneumoperitonitis, and in other pathological conditions where gas occurs in the tissues. Exceptionally it may cause pus-formation.* This bacillus, or the gas formed by it in the organs of human cadavers, appears to have furnished the basis for some of the cases in which death has been ascribed to the entrance of air into the veins during life. It is the same as the organism described by E. Fränkel as Bacillus phlegmonis emphysematosæ.

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*Welch. *Philadelphia Medical Journal.* August 4, 1900.

Bacillus Edematis Maligni (French, *Vibrion septique*).—A bacillus about 1 μ in breadth, 2 to 10 μ in length, which may form threads, having rounded ends when occurring singly. It is motile, having flagella at the sides and ends. It forms spores, and may bulge at the center in consequence of the spores formed there. It retains the stain by Gram's method. It is a strict anaerobe. It grows at ordinary temperatures, but better in the incubator. It liquefies gelatin and blood-serum. The colonies in gelatin are spherical and appear like little bubbles. It grows well upon agar. Gas may be produced in these media.

It is found in garden-earth, street dirt and in putrefying organic material. It is pathogenic to rabbits, guinea-pigs, mice, pigeons and various other animals, including man.* Inoculation results in the production of swelling and edema, spreading from the point of inoculation. Gas may be produced in the tissue. It may lead to wide-spread septicemia.

Bacillus Tetani.—A slim, straight bacillus, with rounded ends, which may form in threads. It is slightly motile. Spores form in culture-media at the end of thirty hours in the incubator. The spores are usually round though it is stated that they are egg-shaped when grown on media containing much sugar or on rice, located at one end, which is swollen, so that in this stage the organism has the shape of a drum-stick. The spores are extremely resistant, and in the dry condition remain capable of germinating under favorable conditions for years.

Earlier statements in regard to the destruction of tetanus spores by steam have generally placed the length of exposure much too short. Among others, Theobald Smith† found the spores much more resistant to sterilization by steam than the statements usually made. He found that in some cases the tetanus spores in his cultures resisted boiling 40 to 70 minutes. This is of importance from the fact that the tetanus spores are so generally distributed in our environment, and that in dressings for wound, in gelatin used for wounds particularly the danger of not properly sterilizing these is imminent. The subcutaneous injection of gelatin to check obstinate hemorrhage has been not infrequently followed by tetanus. Tetanus spores were found by Falcioni* to resist two and one-half hours, but not three hours steaming in a Koch sterilizer. The spores were exposed in this case in 2, 5, and 10 per cent. gelatin solutions. The tetanus bacillus stains by Gram's method. It is a strict anaerobe; it grows in an atmosphere of hydrogen, but not of carbon dioxide. It may sometimes be made to grow very well by Buchner's method. It may be cultivated at the room temperature, but better in the incubator. It grows upon ordinary

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* Gould. *Am. Surgery.* October, 1903.

† *Journ. Am. Med. Assn.* March 21, 1908. pp. 929–934.

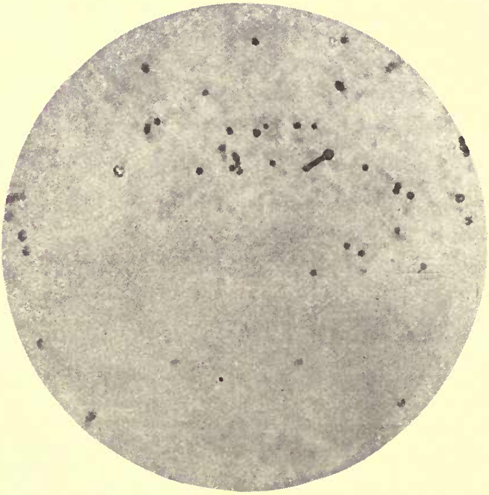

FIG. 79.—One tetanus bacillus (× 1000) with pus cocci. Preparation prepared from a fatal case of tetanus in a human being.

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* *Annali d'Igiene Sperimental.* 1904. Quoted by Smith, *loc. cit.*

culture-media, perferably those containing dextrose. Gelatin is liquefied slowly; the colonies in gelatin present characteristic radiating filaments and look like a bristle brush. It grows on the other culture-media. Gas formation is not pronounced.

This organism appears to be widely spread in external nature, especially in the soil. It is often found in garden-earth and in the feces of herbivorous animals. It is consequently more apt to be encountered in practice in deep, penetrating wounds caused by dirty nails and the like. McFarland claims that it may occur in vaccine virus when this is carelessly prepared, and this would explain those rare cases of tetanus which occur after vaccination.* Tetanus bacilli have been found in gelatin, and it is stated that tetanus has followed the injection of gelatin as a hemostatic. The infection appears usually, if not always, to be introduced through some wound.† Clinically, persons having the disease suffer from spasms of the muscles about the neck and the lower jaw (lock-jaw). The spasms finally become general.

Inoculation with a pure culture produces tetanus in a great many animals. Horses and guinea-pigs, are very susceptible, mice, rabbits, rats are less so in the order named. Sheep, dogs, pigeons and chickens are but little susceptible. The tetanic spasms begin in the vicinity of the point of inoculation and afterward become general. The bacilli are not widely scattered through the body; they occur only in the immediate vicinity of the original lesion, and there are no important macroscopic alterations in the internal viscera.

Tetanus is the type of the purely toxic disease. Its symptoms may be produced in animals by the injection of liquid cultures which have been deprived of their bacteria by filtration. The toxic substance appears not to be a ptomaine, as was at first supposed, and its exact nature is not determined.

Journal Medical Research. Vol. VII. 1902.

† Wells Fourth of July Tetanus. *American Medicine.* June 13, 1903.

Cernovodeanu and Henri* found that the toxin is conveyed by the blood. For if the veins of the part are cut, the occurrence of symptoms is retarded to the same extent as by section of the nerve. After section of the vein and ligation of the muscles, tetanus toxin can be injected into the limb without causing tetanus. That part of the sciatic nerve lying in the extremity of the limb in which the veins and muscles are tied absorbs the toxin.

The poison is tremendously powerful (see page 189). It acts as an excitant to the motor cells of the central nervous system, especially the spinal cord. Bolton and Fisch have shown that horses used for the preparation of diphtheria antitoxin may be infected with tetanus, and have tetanus toxin in the blood† even before symptoms of tetanus are observed in the horse.

The activity of the poison is destroyed by heat and by direct sunlight; various chemicals diminish its intensity.

As shown by Noguchi,‡ the effects of certain photodynamic substances upon ferments, toxins, and protoplasma have been quite extensively studied. Flexner and Noguchi§ repeated and extended the work of others in this direction upon tetanus toxin, and found that eosin in the strength of 1 per cent. solution destroys tetanospasmin quickly in the dark. When eosin and tetanus toxin were injected at different places in the body but simultaneously delayed the onset of the symptoms and porlonged the course of the disease though it did not actually prevent the fatal termination. Noguchi|| found that the injection of eosin "gelb" is more powerful than eosin "rein." The first effect on the organism is to suppress sporulation and

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* Comptes Rend. Soc. Biol. V. XLII. p. 812. May 4, 1907. Abst. *Bull. de l'Inst. Past.* Vol. V., No. 18, Sept. 30, 1907. p. 804.

† *Transactions of the Association American Physicians.* 1902.

‡ *Journ. Exper. Med.* Vol. I. 1906. pp. 252–267.

§ *Journ. Exper. Med.* Vol. VIII. 1906. pp. 1–7.

|| *Loc. cit.* Vol. No. 3. May 25, 1907. pp. 291–297. Eben. pp. 281–290.

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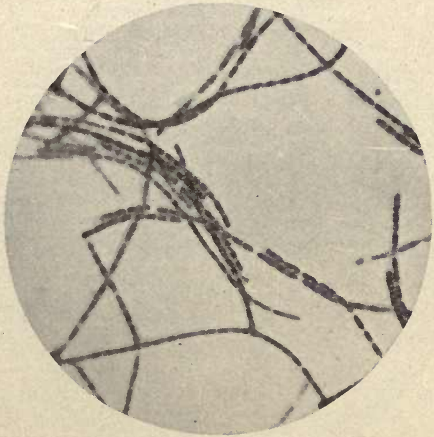
to increase thread formation. The toxin-producing power and virulence of the tetanus bacillus is not permanently effected by contact for a long period with eosin, and cultivation in media containing eosin does not effect these properties permanently. Eosin prevents the germination of spores in the animal body, and it causes the bacilli to degenerate and disappear when injected repeatedly at the seat of the tetanus inoculation. Ungerminated tetanus spores remain alive at the point of injection in eosin-treated animals, and may germinate when introduced into a different location in the same animal and cause tetanus.



FI–. 80.—Anthrax bacilli, from a pure culture.* (X 1000.)

Antitoxin for tetanus has been prepared according to the principles employed for antitoxins in general. It has not proved very markedly successful as a curative agent; but as a prophylaxis, where all patients are treated who have deep, dirty wounds, and in a similar way in veterinary practice, it has undoubtedly proved of value. Unfortunately the disease is seldom suspected until a relatively large amount of toxin has formed and begun to manifest its action in the patient's body.*

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*The culture was derived from a case of malignant pustule in a tanner. The lesion was excised promptly, and the patient recovered.

Bacillus Anthracis.—This is the largest of the pathogenic bacteria with the exception of the spirillum of relapsing fever, which is longer but more slender. The bacillus of anthrax is about 1.25 μ broad, and from 3 to 10 μ long. Bacillus aerogenes capsulatus is of about the same size. The anthrax bacillus often forms long threads. A capsule is sometimes present. It is not motile. It forms spores, which are placed in the centers of the bacilli. The spores form only in the presence of oxygen; they do not appear in the body of an infected animal during life. Anthrax spores are the most resistant of all pathogenic bacteria; they have been known to withstand boiling for more than half an hour †, 5 per cent. carbolic acid

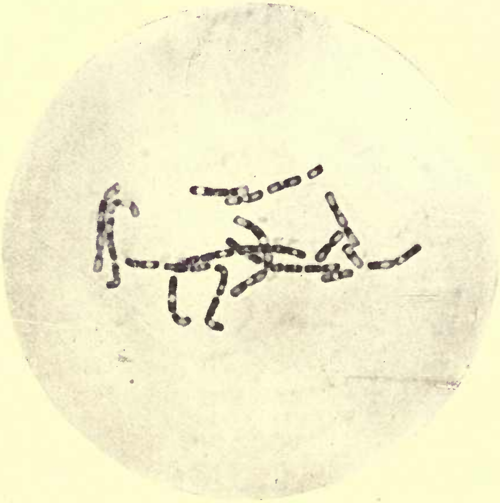

FIG. 81.—Anthrax bacilli, showing spores. (× 1000.)

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*Moschkowitz. *Studies, Department Pathology. College Physicians and Surgeons.* New York. Vol. VII. 1899–1900. *Annals of Surgery.* p. 442. 1900.

† V. A. Moore. *Infectious Diseases of Animals* 1906.

for forty days, and 1–1000 bichloride of mercury for nearly three days. The anthrax bacillus is aërobic, although not strictly so. It stains by Gram's method. It grows at the room temperature, but better in the incubator. It liquefies gelatin and coagulated blood-serum. Colonies in gelatin seen under a low power display numerous, irregular, fine, hair-like projections; stab-cultures in gelatin also present fine projections passing from the needle-puncture into the solid gelatin. It grows on the ordinary culture-media; the growths are usually whitish. Cultures on potato kept in the incubator are favorable to the development of spores. Milk is coagulated and and later peptonized.

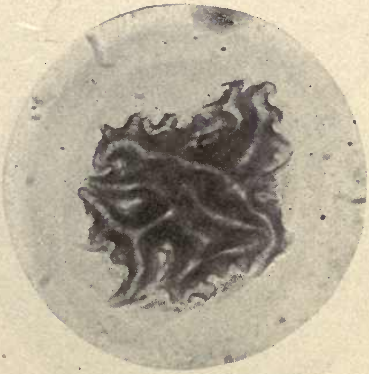

FIG. 82.—Colony of anthrax bacilli (low power), from an impression preparation stained with methplene blue.

It is pathogenic for mice, guinea-pigs, rabbits, cats, certain carnivora and a great many other animals; it is also specially pathogenic for sheep and cattle. Rats and pigeons are quite resistant, but not entirely immune; dogs and frogs are not susceptible, or but slightly so.

Anthrax is a disease which occurs spontaneously chiefly in cattle and sheep. It is commoner on the continent of Europe and in Siberia than in America. In susceptible animals inoculated with virulent cultures of the anthrax bacillus septicemia is produced. Large numbers of the bacilli are found in the blood, and may be crowded together in the capillaries of the liver and kidneys. Men are occasionally affected, especially those whose occupation brings them in contact with cattle or with the hides and wool of animals that die of the disease. The infection usually occurs through wounds of the skin, where it produces a localized inflammation known as malignant pustule. Anthrax of the lungs or "wool-sorter's" disease may be acquired by inhalation of material containing the spores of the bacilli. Infection by way of the intestine occurs occasionally but is less common. Laboratory workers engaged in studying the anthrax bacillus have been accidentally infected in a number of instances.

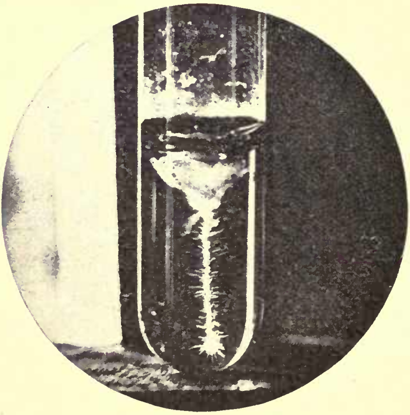

FIG. 83.—Bacillus of anthrax. Stick-culture in gelatin.—(*Günther.*)

The anthrax bacillus, owing to its large size, was the first of the pathogenic bacteria to be recognized, and its study has furnished the basis for much of our knowledge concerning the infectious diseases. It was for anthrax that Pasteur developed the idea of making a protective vaccine, shortly after he had produced a similar vaccine for chicken cholera. There is considerable danger to the inoculated animals attending the use of anthrax vaccines. There is always more or less loss of the animals from anthrax vaccination itself and danger of spreading the disease.

In order to obtain material free from spores the blood of an animal which has recently died of anthrax is taken, because anthrax spores do not form in the living body. Cultures made in bouillon are kept at a temperature of from 42° to 43° C. At this temperature the virulence of the anthrax bacillus becomes gradually diminished. In time the virulence is so far diminished that rabbits will survive inoculation, and eventually also mice and guinea-pigs, which are extremely susceptible to anthrax. Small doses of a culture of extremely weak virulence are given to the animals which it is desired to protect, like cattle and sheep, and subsequently a somewhat more virulent culture is employed.* The method is never used in human beings.

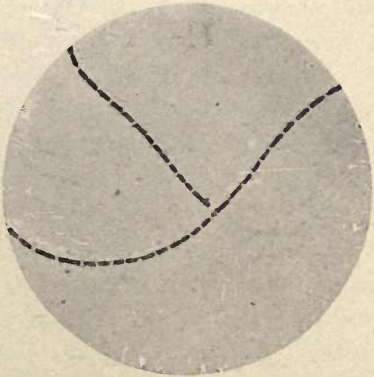

FIG. 84.—Anthrax bacilli with square or slightly concave ends sometimes seen; fuchsin stain. (× 1000.)

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*For details as to the results of this method see V. A. Moore. *Infectious Diseases of Animals.* 1906.

Bacillus Influenzæ.—A small bacillus, 0.2 to 0.3 μ by 0.5 μ, with rounded ends. It does not form spores, is not motile and is decolorized by Gram's method. It is aërobic, grows only in the incubator, and upon media containing hemoglobin. The medium is prepared by smearing sterile blood over the surface of a tube of agar. Fresh, uncoagulated blood may, with care, be mixed with melted agar sufficiently cooled; the mixture may be poured into tubes and slanted; the tubes should be tested in the incubator before using. The blood of some animals, as the pigeon and rabbit, may be used instead of human blood.* The colonies are small and transparent, looking like little drops of water, not becoming confluent.

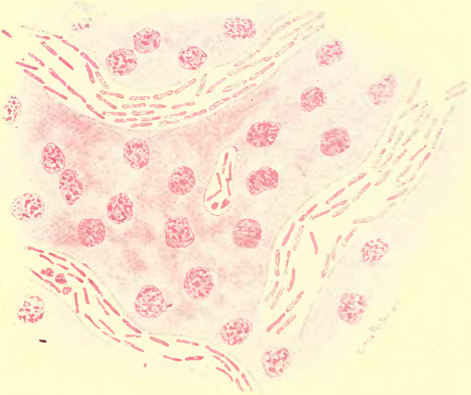

FIG. 85.—Anthrax bacilli in the capillaries of the liver of a mouse, sketched from a section stained with fuchsin.

Of a large number of bacilli, the majority are destroyed in twenty-four hours or less by drying. They die out in a similar manner in water. Experiments upon animals up to this time are not conclusive. For diagnostic purposes, the sputum should be carefully collected in a sterile bottle. If the particles of sputum are likely to have become contaminated, rinse in sterile water. Inoculate on ordinary agar and on blood-agar. The influenza bacillus should grow only on the blood-agar and have the other characters above mentioned. Any organism that grows on both the ordinary and the blood-agar must be rejected. As far as is known, this organism attacks spontaneously only human beings. It probably does not grow outside the body in nature. In cases of influenza it is found in the mucous discharges and in the bronchi and longs. It is the predominating organism in some cases of bronchitis.* According to Canon, the bacilli may sometimes be found in the blood. Wollstein† found the influenza bacillus in the throats and nasal secretion in a number of cases in children suffering from other diseases than influenza, but failed to find it in the normal children. She concludes that the organism is present only in cases where the air passages are affected. In cases where it has been found in apparently healthy individuals there should be a careful inquiry into the previous history, since the influenzea bacillus may persist for a long time. Nevertheless, apparently healthy individuals may be carriers of the organism. She further concludes that there is no justification for the term *pseudoinfluenza* bacillus, and regards the organisms described by others as such, to be merely variations of the same organism.

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*_Centralblatt für Bakteriologie._ Bd. XXXII. Original. p. 267.

Pertussis Bacillus.—Wollstein‡ isolated an organism from the sputum of cases of pertussis which resembled the influenza bacillus in its refusal to grow upon any but hemoglobin containing media. Morphologically it was slightly larger than the influenza bacillus. Wollstein resorted to the Kitasato method of washing the sputum in several changes of sterile water before plating. This was done on agar to which

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*See Lord. *Boston Medical and Surgical Journal* December 8, 1902.

†*Journ. Exp. Med.* Vol. VIII. 1906. pp. 681–691.

‡*Journ. Exper. Med.* Vol. VII. 1905. pp. 335–342.

placental blood was added. She describes the organisms as short, plump ovoid cells. Gram, negative. Colonies on agar transparent, dew-drop-like, surrounded each by a hemolytic zone.

Bacillus Diphtheriæ (Klebs-Löffler).—A straight or slightly curved bacillus, usually 1.2 to 2.5 μ in length, with rounded or slightly pointed ends, remarkable for showing irregularities of form, sometimes being club-shaped or spindle-shaped; branching forms have been found.* It is not motile and does not form spores.

There are two principal forms: One short and relatively plump which takes a very intense uniform stain; the other, that presented in the accompanying photograph, Fig. 81, shows the irregularities mentioned. This irregular form shows sharply marked, intensely stained portions alternating with clear unstained bands running across. This alternation of stained and unstained areas is often quite symmetrical in the rods, giving them the appearance of being striped at almost regular intervals. At other times the stained portion is at one or both of the swollen, club-shaped ends. With methylene-blue the stained portions often appear distinctly red. Considerable practice is necessary to acquire the familiarity required to make diphtheria diagnoses.

It is best stained with watery solutions of the aniline dyes, especially Löffler's alkaline methylene-blue. Very characteristic pictures are obtained by the method of Neisser:

SOLUTION NO. 1.

Methylene-blue,	1
Alcohol (96 per cent.),	20
Distilled water,	950
Glacial acetic acid,	50

SOLUTION NO. 2.

Bismarck brown,	1
Boiling distilled water,	500

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*Hill. *Journal Medical Research.* Vol. VII. 1902.

Stain the cover-glass preparation which has been fixed in the flame in No. 1 one to three seconds; wash in water; stain in No. 2 three to five seconds; wash in water; mount as usual. The body of the bacillus is stained pale brown, with dark blue spots, especially at the ends (Fig. 87). In regard to the Gram stain, some strains retain the stain while others do not. Hamilton and Horton* found that of 18 cultures isolated by them, 11 were Gram negative, 7 were Gram positive.

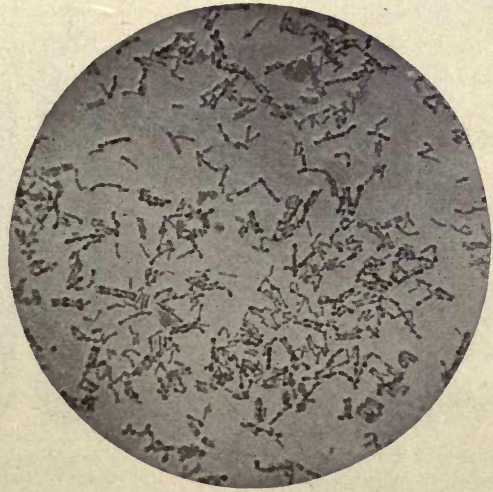

FIG. 86.—Bacillus of diphtheria. (X 1000.)

The diphtheria bacillus is peculiar in staining irregularly; certain spots stain more sharply than other portions, and darkly stained spots are likely to occur at the ends. It is a facultative anaërobe. It grows most rapidly in the incubator, and slowly, or not at all, below 20° C. Gelatin is not liquefied. It may be cultivated on various alkaline culture-media, but grows best on Löffler's blood-serum mixture (page 75). On this medium the growth consists of small white or cream-colored, slightly elevated colonies, which may become confluent. The morphology of the bacillus is most characteristic when it is cultivated on blood-serum. It also grows upon glycerin-agar. On potato it grows only if the potato is first treated with soda solution (1 per cent.) so as to neutralize the acidity of this medium.* In alkaline bouillon containing dextrose or muscle-sugar the reaction becomes acid in forty-eight hours.† The reaction of the bouillon subsequently becomes alkaline. The growth may form a pellicle over the surface of the bouillon. It has also been successfully cultivated on various media to which egg-albumen has been added.

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* *Journ. Infect. Dis.* Vol. III. 1906. p. 132.

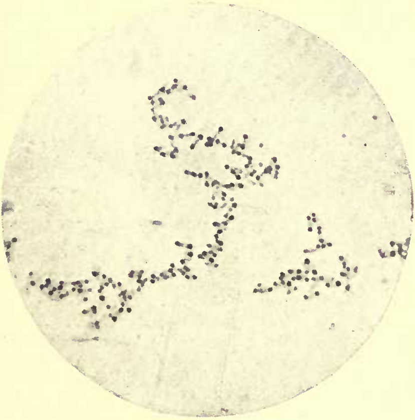

FIG. 87.—Bacillus of diphtheria stained by Neisser's method. (× 1000.)

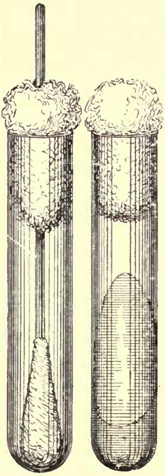

FIG. 88.—Swab and culture-tube used in the diagnosis of diphtheria.

It is killed by a moist heat at about 60° C. in ten minutes. It is very resistant to drying.

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* Günther. *Loc. cit.*

† Kolle and Wassermann. *Loc. cit.* p. 769–770.

Bacteriological Diagnosis of Diphtheria.—In many large cities the bacteriological diagnosis of diphtheria is undertaken by boards of health. The methods used differ somewhat in detail, but are similar in the main, and are based upon the procedure devised by Biggs and Park for the Board of Health of New York City. Two tubes are furnished in a box. The tubes are like ordinary test-tubes, about three inches in length, rather heavy and without a flange. Both are plugged with cotton. One contains slanted and sterilized Löffler's blood-serum mixture (Fig. 88); the other contains a steel rod, around the lower end of which a pledget of absorbent cotton has been wound. These tubes containing the swabs are sterilized, and it would seem that an efficacious method would be to sterilize them first in the autoclave and subsequently in the dry sterilizer. The swab is wiped over the suspected region in the thoat, taking care that it touches nothing else, and is then rubbed over the surface of the blood-serum mixture. The swab is returned to its test-tube and the cotton plugs are returned to their respective tubes. The plugs, of course, are held in the fingers during the operation, and care must be taken that the portion

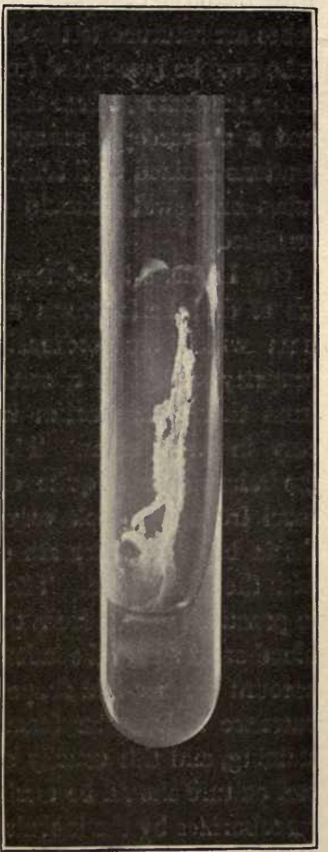

FIG. 89.—Bacillus of diphtheria, culture on glycerinagar.

of the plug that goes into the tube touches neither the finger nor any other object. The principles, in fact, are the same as those laid down in general for the inoculation of culture-tubes with bacteria (see page 78). In board of health work these tubes are returned to the office. When it is desirable, a second tube may be inoculated from the swab. The tubes are placed in the incubator, where they remain for from twelve to 15 hours and a microscopic examination is then made of smear preparations stained with Löffler's methylene-blue. After use the tubes and swabs should be most carefully and thoroughly sterilized.

On Löffler's blood-serum kept in the incubator the bacillus of diphtheria grows more rapidly than the other organisms which are ordinarily encountered in the throat, a property which to a certain extent sifts it out, as it were, from them, and makes its recognition with the microscope easy in most cases. The appearance of the bacilli under the microscope is quite characteristic. Colonies of streptococci frequently look very like those of the bacillus of diphtheria but those two are easily distinguished from each other with the microscope. The diagnosis of the diphtheria bacillus in practice is made from the character of the growth upon the blood-serum and the microscopical examination, taking into account the size and shape of the bacilli, with the frequent occurrence of irregular forms and the peculiar irregularities in staining, and this usually suffices; but in doubtful cases a second culture should be made from the throat, and if necessary corroborates by the inoculation of a guinea-pig.

The very large number of examinations that have been made by various boards of health have shown that the diphtheria bacillus may persist in the throat for a long time—occasionally several weeks after the patient has apparently recovered; also that diphtheria bacilli are occasionally found in the throat when there is an inflammatory condition without any pesudomembrane, and that they not only appear in an apparent healthy throat, especially in hospital nurses and in children who have been associated with cases of diphtheria, but also in those who have had no tracable contact with diphtheria cases.* It has been found that bacilli sometimes occur in the throat which have all the morphological and cultural properties of the diphtheria bacillus, but which are devoid of virulence when tested upon animals. Such diphtheria bacilli have frequently been called *pseudodiphtheria bacilli.* A bacillus closely resembling the diphtheria bacillus, but without virulence, has been found in xerosis of the conjunctiva. It is called the *xerosis bacillus.* If not a transformed diphtheria bacillus, it is at least closely related. The diphtheria bacillus is subject to wide variations in morphology, so that, in dealing with unknown cultures where the forms are not characteristic and injection into animals is without result, it may be difficult to decide whether or not the organisms are diphtheria bacilli. Consequently another view with regard to pseudodiphtheria bacilli has arisen. While recognizing that non-virulent diphtheria bacilli occur, it is also claimed that a distinct *pseudodiphtheria* bacillus exists, different from the diphtheria bacillus, though resembling it. It is shorter, stains more evenly, shows no polar granules by Neisser's method of staining, does not produce acid in dextrose-bouillon, and is not pathogenic to animals and does not produce diphtheria toxin. It is found occasionally in the nose and throat and has no connection with diphtheria, according to this view.† But there are some who hold that there is no pseudodiphtheria bacillus, and that the

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*Sholly. *Journ. Infect. Dis.* Vol. IV. 1907. pp. 337–346.

† The different sides of this question will be found fully discussed by the following: Wesbrook, Wilson and McDaniel. *Transactions of the Association American Physicians.* 1900. Gorham. *Journal Medical Research.* Vol. VI. 1900. A. Williams. *Ibid.* Vol. VIII. 1902. Denny. *Ibid.* Vol. IX. 1903. Alice Hamilton. *Journal of Infectious Diseases.* Vol. I., No. 4. 1904. Graham Smith. *Journal of Hygiene.* Vol. IV. 1904.

organism so called it merely a more or less modified form of the diphtheria bacillus.

At a meeting of the Association of American Pathologists and Bacteriologists, Perkins* described two organisms, or one organism with variations of characteristics, which he came across in routine examinations of cultures from throats of persons suspected of having diphtheria, and which he regards as likely to lead to error. The points of difference from the diphtheria which are given below would seem, however, to make a differentiation not very difficult with proper care. Although resembling the bacillus of diphtheria bacillus, it differs in having a greater regularity in outline than the latter, and in fact that this regularity persists after treatment with acetic acid. The chromophylic granules take a deeper stain than in the diphtheria bacillus. The organism is motile and forms spores. In old cultures there is distinct thread formation, with true, but inconstant branching in cultures over a month old. Gelatine is slowly liquefied. To guard against error it would seem necessary for those who make routine examinations for diphtheria merely to test the organisms which resemble diphtheria bacilli for motility in order to avoid the possibility of mistaking this organism of Perkins for the genuine diphtheria bacillus. The formation of spores after twenty-four hours would also enable one to distinguish the Perkins organism from the true diphtheria bacillus.

Persons who harbor the diphtheria bacilli in their throats whether they show any clinical symptoms or no should be regarded as a menace to those around. Their throats should be actively treated with suitable antiseptics and water, and frequently examined for the presence of the organism. It is probable that in this way such persons can be quickly rendered harmless to those about them.

The diphtheria bacillus is pathogenic for guinea-pigs, rabbits, cats, chickens, pigeons and to a somewhat lesser extent, for dogs, goats, cattle and horses.† When it is injected into them it produces a toxemia. In the guinea-pig, which is especially susceptible, local inflammation results, and death occurs usually in two or three days. The bacilli are found to be confined to the vicinity of the wound, and not usually to be disseminated throughout the whole body. The death of the animal, therefore, is due to the poisons elaborated by the diphtheria bacilli—either poisons introduced at the original injection, or substances produced by the bacilli which may have multiplied in the animal's body. The internal viscera, especially the liver, often exhibit small areas consisting of necrotic cells; a transudation of serum takes place in the great serous cavities, and the lymph-nodes are swollen. A genuine diphtheritic membrane may be produced on the trachea of a young kitten by rubbing into it a part of a culture of the diphtheria bacillus.

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*Personal communication to Dr. Williams.

†Park. Pathogenic Bacteria and Protozoa. New York and Philadelphia. 1905. p. 192.

As is well known, the pseudomembranous affection produced by the diphtheria bacillus in man is generally seen in the larynx and pharynx. Membranous rhinitis is also caused by the diphtheria bacillus. On the other hand, pseudomembranous affections of the larynx and pharynx indistinguishable from diphteria except by bacteriological examination may be produced by streptococci.* Pseudomembranes occurring in the throat during scarlet fever and measles may be due to the diphtheria bacillus, but are more often caused by streptococci. The affection known as membranous croup is usually diphtheria of the larynx produced by the diphtheria bacillus. The diphtheria bacillus is a rare cause of puerperal fever. Although the uninjured skin is not attacked by the diphtheria bacillus, it may be present in pseudomembranes on wounded surfaces, usually in connection with diphtheria in the throat. Most pseudomembranes formed upon wounds of the skin are produced by other bacteria than the diphtheria bacillus, as is also the case with the pseudomembranous inflammations of the intestines and bladder. Although such inflammations are often called "diphtheric," it must be remembered that the ex-

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*Bissell. *Medical News*. May 31, 1902. *American Journal Medical Sciences*. February, 1903. Review of Work of Massachusetts Boards of Health.

pression is used in an anatomical sense, meaning that a fibrinous pseudomembrane has formed, extending deeply into the tissues, which is not necessarily caused by the diphtheria bacillus.

The diphtheria bacillus is usually found associated with other pathogenic bacteria in cases of diphtheria. The pus cocci and the pneumococcus very frequently complicate the disease. It seems that the diphtheria bacillus may be either the cause of the primary disease and prepare the way for secondary invasion, or on the other hand it may follow or accompany a primary infection of some other organism. In other words, cases starting as diphtheria may, and usually do become complicated by pus coccus invasion, or cases starting pus coccus infection or as measles or scarlet fever or pneumonia may be complicated by a secondary infection with the diphtheria bacillus

In cases of diphtheria in man,* the diphtheria bacillus is generally found limited to the vicinity of the pseudomembrane, and at autopsies it is not usually found in the internal viscera, excepting in the lungs, where diphtheria bacilli may or may not be present when diphtheria is complicated with bronchopneumonia. The general symptoms of the disease, including the paralysis which sometimes follows it, are due to the toxins produced by the bacilli in the throat.

Diphtheria Antitoxin.†—It is necessary first to obtain the toxin produced by diphtheria bacilli in a concentrated form. For this purpose virulent diphtheria baccilli are cultivated in alkaline, sugar-free bouillon, in flasks plugged with cotton, exposing a large surface to the air. Park ‡ finds that the presence of muscle-sugar makes no difference, provided the broth is made sufficiently alkaline to neutralize the acid formed by the fermentation of the sugar, and also where the culture employed is a vigorous one.* On the other hand Theobold Smith † finds that the alkalinity of the broth is not the essential point in the production of strong toxin, but that it is necessary to use broth which has been fermented with the colon bacillus to rid it of muscle-sugar. Smith's directions are as follows:

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*For a full study of the lesions of diphtheria see the Monograph of Councilman, Mallory and Pearce. Boston. 1901.

†See articles by Park, A. Williams, Atkinson and T. Smith. *Journal of Experimental Medicine.* Vol. I., p. 164; Vol. III., p. 513; Vol. IV., pp. 373 and 649. *Journal Medical Research.* Vol. IX., p. 173.

‡Park. *Loc. cit.* p. 194.

Beef infusion prepared in the usual way by using 500 grams of finely chopped, lean beef is kept in the cold for 12–24 hours; the juice then expressed; the reaction brought to 1.5 or 2 per cent. acid with normal sodium carbonate solution; heated to 40° C.; inoculated with 30–40 c.c. of a 24-hour bouillon culture of B. coli; and placed in the high-temperature incubator for 16 hours or over night; it is then clarified by adding the white of one egg to every liter of infusion and boiling for 45–60 minutes in the Arnold sterilizer; cooled down and filtered; 2 per cent. of Witte's peptone and 0.5 per cent. of common salt are added and dissolved by gentle heat; the acidity is reduced to 0.8 per cent. with sodium carbonate; 0.1 per cent. of dextrose is added; steamed for or boiled for 20–30 minutes and filtered; distributed into Fernbach flasks in shallow layers, 2.5 ccm. deep and sterilized in the autoclave at 110–115° C. for 30 minutes. The cultures of diphtheria which are employed should form membranes over the medium promptly, leaving a clear fluid beneath. Previously grown beef-broth cultures in test-tubes are used to inoculate the Fernback flasks. The cultures in these flasks become distinctly alkaline to phenolphthalein in 6–8 days at which time the maximum of toxin has been formed. After having been tested for purity with the microscope and by cultures they are treated as follows: "Rendered sterile by the addition of 10 per cent. of a 5 per cent. solution of carbolic acid. After 48 hours the dead bacilli have settled on the bottom of the jar and the clear fluid above is syphoned off or it is filtered through ordinary sterile filter paper and stored in full bottles in a cold place until needed." The filtrate contains the toxin. The toxin is injected into the animal from which the antitoxin is to be obtained in small doses. The dose depends on the strength of the toxin. The aimal usually employed is the horse, which should be healthy; the presence of tuberculosis and glanders should have been excluded by testing with tuberculin and mallein; the possible presence of tetanus should also be considered (see page 330).

The injection is repeated at intervals of about one week, using larger and larger doses, until the animal is able to tolerate a very large dose indeed—as much as 300 c.c., or even more. If the treatment is successful, the general condition of the animal should not suffer. The injections last over a long period—usually about two or three months. The general condition of the animal remaining good, the toleration of these large doses of toxin is presumed to indicate the existence of a concentrated antitoxic substance in the blood.

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* Park. *Loc. cit.* p. 205–206.

† *Journ. Exper. Med.* Vol. IV. 1899. pp. 273–297.

Small quantities of blood may be withdrawn from time to time, and the serum tested for its antitoxic strength. When a satisfactory serum has been attained, the animal may be bled and the serum saved for therapeutic purposes. Through an incision in the skin a trocar is inserted into the jugular vein. The blood is drawn into sterilized flasks with every precaution to insure sterility. The blood is allowed to coagulate and is placed for a time in the ice-chest. The serum is then withdrawn with sterilized pipettes. Small amounts of chemical germicides, as carbolic acid or chloroform, are sometimes added to assist in preserving it. This serum is the so-called antitoxin used in medical practice.

Many methods have been recommended for concentrating antitoxin. Gibson* recommends a modification of the method devised by Pick. Briefly stating and omitting many details, Pick's method consists of precipitating the antitoxic serum with a saturated solution of c.p. ammonium sulphate. Gibson redissolves this precipitate with a saturated solution of sodium chloride, reprecipitates with ammonium sulphate or better with dilute acetic acid and dializes in running water over night, neutralizes the acetic acid if this has been used, and dializes in running water for two days longer. This preparation is said to possess many advantages. For one thing there is said to be no rash following its use as with so many preparations of antitoxin.

Since antitoxin is not obtained as a pure chemical substance, and consequently cannot be weighed and measured as other theraputic preparations, an arbitrary standard to express the potency of the serum, called an immunity unit, has been devised by Behring and modified by Ehrlich.† Formerly this unit was taken to be 10 times that amount of antitoxic serum which just neutralized 10 fatal doses of toxin for guinea-pigs weighing 250 grams. In other words, the exact amount of a certain toxin required to kill a guinea-pig weighing 250 grams in four days having been determined by inoculating a number of guinea-pigs, ten times this amount was put into each of a number of test-tubes, and the antitoxin to be tested was added, a slightly different amount to each tube of toxin. The contents of each tube was then injected into a separate guinea-pig.

If any of the animals survived, the amounts of antitoxin in the tubes with which they had been inoculated having been noted, the smallest of these amounts —*i. e.*, the smallest amount found necessary to neutralize the toxin—was regared as one-tenth of an antitoxic unit. It was naturally assumed that 10 times this amount of antitoxin would neutralize 100 fatal doses. This, however, was found not to be the case (see Immunity, page 223). So the revised standard now employed in Germany, France, America and other countries is the unit recommended by Ehrlich. This consists of comparing the antitoxin

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**Journ. Biol. Chem.* Vol. I. Jan., 1908. pp. 161–170. Abstr. in *Bun. de l'Inst. Past.* Vol. IV., No. 9. 1906. p. 426.

†Rosenau. Immunity Unit for Diphtheria Antitoxin. Public Health and Marine Hospital Service. Bulletin 21. 1905.

to be standardized with antitoxin specially prepared by Ehrlich for the purpose. This antitoxin of Ehrlich is supplied to the various public and private institutions where antitoxin is prepared, and is carefully standardized against very fresh toxin, which therefore contains little toxoid.

The Ehrlich standard antitoxin is really used in the first place to determine the strength of a given toxin, which in turn is used to determine the value of antitoxin to be standardized. The actual method is to mix varying amounts of the toxin to be tested each with one unit of the standard antitoxin, and that mixture which just suffices as proved by experiment to kill a 250-gram guinea-pig in three or four days is designated L + (see Immunity, page 224); the mixture which is just neutral is called LO. That amount of antitoxin which just neutralizes L + contains one antitoxic unit according to this method of standardizing.

The injection of guinea-pigs with antitoxin serves the double purpose of determining the potency of the antitoxin and also of determining the presence or absence of pathogenic substances, such as tetanus toxin.

It has been found possible to prepare antitoxin of a high degree of concentration, so that 500 to 1500 units may be contained in a quantity of serum which it is practicable to give at a single hypodermic injection. The large volume of statistics that have been collected from hospitals and from physicians in private practice indicates that the use of diphtheria antitoxin has effected a very great reduction in the mortality from diphtheria. Aside from the curative value of diphtheria antitoxin, it is of value as a preventative when administered to persons who are exposed. Where a case of diphtheria occurs in a family all the other members beside the patient should be given an immunizing dose of antitoxin—about 500 units suffices. When this is done, the disease is limited to the one case.

Bacillus Fusiformis.*—Under this name an organism was isolated by Vincent and claimed by him to be the cause of certain infectious pseudomembranous ulcerations. Vincent's observations have been more or less corroborated by others, but the crucial tests of cultivation and inoculation do not seem to have been made successfully to prove the pathological significance of the organism.

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* Vincent. *Ann. del' Institute Pasteur.* 1896. p. 488.

The bacillus is described as non-motile, varying from 6–8 μ to 10–20 μ in length. It appears mostly or exclusively to be mixed with other bacteria. Bernheim observed the organism in ulcerative stomatitis mixed with spirochetæ.

Bacillus Tuberculosis.—A slim bacillus with rounded ends 1.5 to 4 μ in length. It very frequently presents a beaded appearance, owing to its being dotted with bright, shining spots.

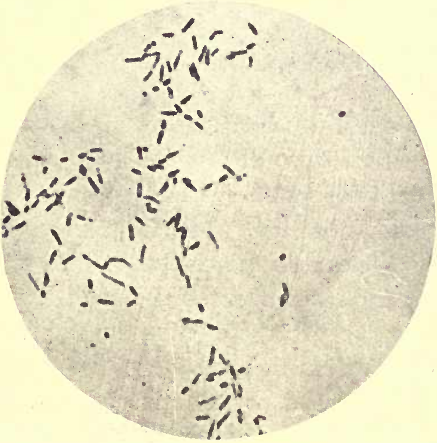

FIG. 90.—Bacillus tuberculosis, from a pure culture. (× 1000.)

Branching forms have been described. The tubercle bacillus is considered by some to be a member of the actinomyces group. It is not motile. It has not been proved that spores are formed; nevertheless certain structures, like caseous lymph-nodes, have been shown to be capable of infecting guinea-pigs with tuberculosis, although tubercle bacilli could not be demonstrated in them with the microscope. This makes it seem possible that the organisms were present as spores which eluded the microscopical examination. The tubercle bacilli stain with the ordinary aniline dyes and by Gram's method, but they do not take the stains as readily as most other bacteria, and require somewhat longer exposure to the dye than other bacteria, on warming of the stain. When once stained, however, with aniline-water dyes or carbol-fuchsin, they are not readily decolorized by acids and alcohol, which fact distinguishes them from all other known bacteria excepting the leprosy bacillus, the smegma bacillus, possibly the bacillus of syphilis (Lustgarten), and certain bacilli found in milk, butter and cow-dung and on various grasses. All of these may resist decolorization by acids or alcohol, and some resist both. They must always be kept in mind in making a diagnosis of tuberculosis. (See pages 33 and 36.) In examining sputum it is particularly important to bear in mind that acid-proof bacilli, resembling tubercle bacilli, have been found in rare cases in gangrene of the lung. But the organisms found in these cases are longer than tubercle bacilli, as a rule, and branch more often, besides being less resistant to decolorization.* The tubercle bacilli appear to owe their peculiar staining properties to fatty substances contained in the bodies of the bacilli. In stained preparations the bacillus usually appears very distinctly beaded, owing to the presence of stained areas which alternate with unstained areas; these unstained areas have been considered by some to be spores.

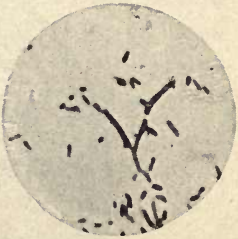

FIG. 91.—Branching form of tubercle bacillus from a culture. (× 1000.)

The Bacillus tuberculosis is aërobic. It requires certain special media for its cultivation (see below), and it does not grow so readily when it is first inoculated from tuberculous material from man or lower animals as it does subsequently.

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* Ophüls. *Journal Medical Research.* Vol. III. 1902. Ohlmacher. *Journal American Medical Association.* 1901.

But after it becomes accustomed to the artificial environment, it may be readily cultivated on a number of different media, though its growth is always relatively slow as compared with that of many other bacteria. It does not grow at a temperature below 29° C., and the best temperature is around 38° C. It cannot, therefore, be cultivated upon gelatin even if this were a suitable medium otherwise. It grows well upon blood-serum, where the growth becomes visible in from ten to fourteen days in the incubator. It forms a dry, mealy, scaly mass, elevated above the surface, of a grayish-brown color. It also grows upon glycerin-agar; or glycerin-bouillon, on which it forms a pellicle; upon potato; upon milk containing 1 per cent. of agar and upon coagulated egg (see page 76). It is important to have the medium moist. It can be cultivated from tuberculous sputum only with great difficulty. It is best to obtain it from the tissues of an animal that has died of tuberculosis, where the

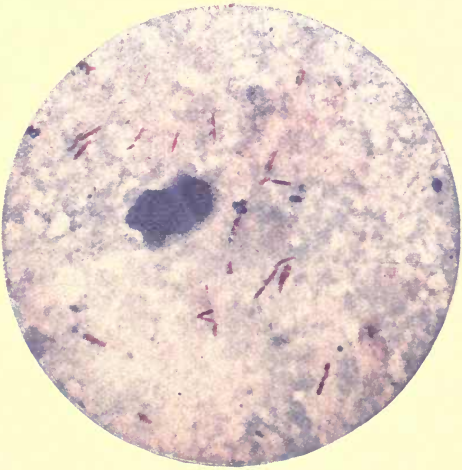

FIG. 92.—Bacillus tuberculosis in sputum, stained with carbol-fuchsin and methylene-blue. Photomicrograph in two colors. (× 1000.)

tubercle bacilli may be found unmixed with other bacteria. Pieces of tissue should be taken with the precautions necessary to avoid contamination, and should be broken up and rubbed over the surface of the medium. The tubes must be closed with sealing-wax, paraffin or rubber stoppers, or covered with rubber caps, to prevent drying in the incubator. If rubber caps are used, they should first be left in 1–1000 bichloride of mercury for an hour, and the cotton plug should be burned before putting on the rubber cap. A number of tubes should be inoculated, using rather large particles of the tuberculous material. Among the tubes inoculated, some are apt to show no growth. After the organism has once been grown upon a culture-medium it may be propagated with less difficulty.

The statement in regard to the action of germicidal agents in general may be repeated here with regard to their action upon the tubercle bacillus. The results of laboratory experiments to determine the effects of the various agents upon the tubercle bacillus cannot with safety be absolutely relied upon in practice to destroy the bacilli. The measures employed in practice should in all cases be much more drastic than would be indicated as just sufficient by the results of laboratory experiments. Absolute destruction by fire should be resorted to whenever this is feasible, and next to this sterilization in the autoclave at 115 to 120° C. Chemical disinfectants are uncertain, particularly in the disinfection of sputum, feces and the like. The following statements in regard to the effects of various germicides are, therefore, of more theoretical than of practical interest.

The bacilli are very resistant to drying. They remain alive for about two months when kept dry. They are also more resistant to the destructive action of heat when dry than when moist. They have been known to remain alive after one hour's heating at 100° C. when dry; but when they are exposed in water, milk, beef-broth or other fluids they are killed at 55° C.

in four hours, at 60° C. in 30 minutes, at 65° C. in 15 minutes, at 70° C. in 10 minutes, at 80° C. in five minutes, at 95° C. in one minute. These temperatures are of importance in the matter of the sterilization of milk, since it is important that milk should be heated as little as necessary. Theobald Smith showed that the scum which forms on the surface of milk when it is heated protects the tubercle bacilli which are in the scum from the effects of the heat. Sunlight destroys the bacilli quickly, but in practice the protective coating of sputum or other material may interfere with the action of the sunlight. In fact the protection afforded by the sputum may operate in all cases where the bacilli are sought to be destroyed. It is not destroyed always by the gastric juice as is seen in those cases referred to below in which the bacilli are found in the feces of persons affected with phthisis, and who swallow their sputum. It has furthermore been shown by direct experiment, as well as by the occasional occurrence of primary intestinal tuberculosis, however rare, this may be comparatively speaking. In fact the view that the bacilli are not destroyed by the gastric juice is the basis for Behring's contention that all forms of tuberculosis are acquired by ingestion of the bacilli. They are destroyed in sputum by carbolic acid in the proportion of equal parts of a five per cent. solution of the carbolic acid to the amount of sputum. The fumes from 4 pounds of burning sulphur to each 1000 cubic feet of air space kills the tubercle bacillus in 8 hours provided the bacilli are freely exposed and the atmosphere is kept moist.* Formaldehyde is quicker but not more efficient in the proportion of 10 ounces of formalin to 1000 cubic feet of space.†

It is not known to grow, except in artificial cultures, outside of the animal body. It is the cause of tuberculosis in man. It produces tuberculosis in apes, cows, hogs, sheep, horses,

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*Park. *Loc. cit.* p. 290.
†*Ibid.*

rabbits, guinea-pigs, cats, field-mice and occasionally in other animals. Guinea-pigs and rabbits are extremely susceptible. A guinea-pig inoculated with tuberculous sputum (provided it does not die of septicemia, due to the pyogenic micrococci which are frequently present in sputum) will present a swelling of the neighboring lymph-nodes in the course of two to four weeks, and will die as a rule in from four to eight weeks, although the time may be longer.

Tuberculosis in cattle (German, *Perlsucht*) is characterized by large, nodular lesions, with a marked tendency to become fibrous, caseous and calcified. The tubercle bacilli of cattle differ somewhat from those of human tuberculosis, as was noted by Theobald Smith.* Whether or not men could be infected with bovine tubercle bacilli has been a question that has been warmly debated in recent years. There seems no longer room for doubt that such infection does take place; also that cattle may be infected with human tubercle bacilli. Bovine tubercle bacilli are more virulent for some animals, as rabbits, than human tubercle bacilli.†

The tubercle bacillus is present in all forms of tuberculosis, in the sputum from the lungs of persons suffering with phthisis, in all tissues affected with tuberculosis, as in the skin in lupus; in tuberculous glands in all situations, in the cervical glands in scrofula, in the mesenteric glands in intestinal tuberculosis; it is present also in the feces in intestinal tuberculosis, in the urine in tuberculosis of the urinary apparatus. Wherever there is a tuberculous lesion in any location in man or in the lower animals, and in the excreta and in the secretions from tuberculous organs the tubercle bacillus is to be found. Not only in such locations but also in certain conditions where there is no lesion. It has been found in the mouth, throat and nose of persons who show no symptoms or signs of tuberculosis, but who associate intimately with tuberculous persons. It is found in the glands of persons dying from other causes

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***Journal Experimental Medicine.* Vol. III., p. 451.

†Theobald Smith. *Medical News.* February 22, 1902. Various bulletins from the Bureau of Animal Industry. Adami. *Philadelphia Medical Journal.* February 22, 1902. Ravenel. *University of Pennsylvania Medical Bulletin.* May, 1902. Lartigau. *Journal Medical Research.* Vol. VI. 1901. Wolbach and Ernst. *Ibid.* XII., p. 295. Theobald Smith. *Ibid.* XIII., p. 299.

than tuberculosis in a very large proportion of cases. Indeed, the statement is made by competent authorities that nearly all persons above the age of 18 years probably have latent tuberculous foci in their bodies.*

In nearly every case which comes to autopsy, whether the subject showed evidence of tuberculosis during life or no, tubercle bacilli may be found in the lymph-glands, if not directly with the microscope at least by the inoculation of susceptible animals with bit of the gland. In this way as many as 90 per cent. and over of autopsies in hospitals have revealed the presence of tubercle bacilli whether there were lesions of tuberculosis or no lesions of tuberculosis. The feces of persons who are suffering from pulmonary tuberculosis may contain the bacilli even in cases in which there is no involvement of the intestine. In such cases the bacilli are swallowed with the sputum, and are discharged from the intestines.

In external nature the tubercle bacillus is found for the most part in the air surrounding tuberculous patients. They are found not only in the dry air attached to floating particles, but Flügge has shown that they are thrown into the air by tuberculous persons in every act of coughing, and that they remain for a long time floating about on the little globules of sputum.

The lesion produced by the tubercle bacilli in the tissues of men and the lower animals is called a tubercle, which in the beginning is a grayish-white area about the size of a millet-seed. In sections of the tissue young tubercles are found to present several different structures. Near the center, one or more very large cells called giant-cells occur. They contain several or many nuclei which are frequently arranged in a crescentic manner at one side of the cell. Tubercle bacilli can sometimes be demonstrated inside of the giant-

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*Günther. *Loc. cit.* p. 472.

cell. Except possibly in the very youngest tubercles, a small area of necrotic tissue will usually be found at the center of the tubercle.

Around the giant-cells and the necrotic area are seen large cells with distinct nuclei which resemble epithelial cells, and are often called epithelioid cells; they are also often termed granulation cells, and represent an attempt at the formation of granulation tissue. But no new-formed blood-vessels, such as are found in granulation tissue as a rule, occur in the tubercle. Tubercle bacilli may also be found among the epithelioid cells. Outside of these epithelioid cells is another layer of small cells called lymphoid cells, which represent leukocytes that have appeared in this situation as a part of the inflammatory reaction excited by the presence of the tubercles. The zone of lymphoid cells may be very indistinct or wanting. Frequently it may be very difficult to make out that the cells are arranged in distinct zones at all, for instead of distinct tubercles, the tubercle bacillus may produce a diffuse form of inflammatory tissue. The cells are imbedded in a matrix consisting of the connective tissue originally belonging to the part, to which some fibrin may be added. In addition to the fact that no new blood-vessels are formed to maintain the nutrition of these newly formed cells, the small vessels included in the tubercle and around it suffer from inflammatory changes. Owing to these causes and to a toxic substance formed by or in the tubercle bacilli, degenerative changes and necrosis take place at the central part of the tubercle. As a result of these degenerative changes the center of the tubercle becomes converted into a dry, yellowish-white, friable mass, resembling dry cream-cheese. Such material is said to be caseous, and the process is called *caseation*. Prudden and Hodenpyl found that the injection of dead tubercle bacilli into animals produced lesions having the histological characters of tubercles, but caseation did not take place.

The small tubercles first formed are called *gray* or *miliary tubercles*. As they become larger they also frequently become confluent. The larger, confluent, caseous tubercles are often called *yellow tubercles*. Swollen tuberculous lymph nodes of the neck are among the manifestations of the condition formerly known as *scrofula*.

Masses of caseous tubercles sometimes undergo softening. In the lungs the discharge of the softened material results in the formation of a cavity. This formation of a cavity in the lungs is frequently, if not usually, accompanied by secondary infection with pyogenic micrococci. Caseous tuberculous masses may become partly calcified. Very often they may be encapsulated by new formed fibrous or scar tissue. It is possible for tuberculosis to become cured for all practical purposes by means of this process. Autopsies on human subjects have shown that such cures not rarely take place, especially in tuberculosis of the lungs occurring over a localized area. The statistics of autopsies vary widely as to the number of persons that at some time of life suffer from tuberculosis, from 25 or 30 per cent. up to much higher figures. When a tuberculous area has become caseous and encapsulated and apparently quiescent, it is possible for it to be excited to renewed activity under suitable conditions, and, owing to the softening and the discharge of infected material into one of the vessels or cavities of the body, a wide-spreading and rapidly fatal tuberculosis may follow.

Tuberculosis may become disseminated throughout the body from a small focus as a starting-point. The tubercle bacilli may travel through the lymph-spaces and affect adjacent tissues, some of them reaching the nearest group of lymph-nodes. In tuberculosis of the lungs it is usual also to find tubercles in the bronchial lymph-nodes, and in tuberculosis of the intestines there is also tuberculosis of the mesenteric lymph-nodes. The disease may travel along the serous surfaces and become widely scattered throughout a cavity like that of the pleura or peritoneum. The bacilli may be expelled on some mucous surface and be carried along it to infect some point farther on, as happens when the larynx becomes infected in tuberculosis of the lung, and when in the same disease tuberculous sputum is swallowed and leads to infection of the intestines. Finally, the infectious material may enter the blood-vessels, especially the veins, and be swept along with the blood-current to become scattered generally throughout the body. In such cases we are likely to have general or *acute miliary tuberculosis*. Almost every organ of the human body may be infected by tuberculosis. Among the most common may be mentioned the lungs, the lymph-nodes, the bones, the intestines, the skin, the meninges, and the serous membranes.

Harbitz* found as a result of his observations that primary tuberculosis of the lymph glands is quite frequent in adults, not only in the thorax, but also in the abdomen, and especially in the cervical nodes, often it is generally distributed in the lymphatic system having extended through years and tens of years.

Infection, as far as we know, is always to be attributed directly or indirectly to some preëxisting case of tuberculosis in man or the lower animals.

The mode of entry into the body of both man and animals is a matter of liveliest dispute; some holding with Flügge that the commonest mode of entrance is by way of inhalation into the lungs; others maintaining with Behring that the entrance is always by the alimentary tract.† Those who hold the latter view have this much to present in evidence that, as Ravenel showed, the tubercle bacillus may pass through the

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**Journ. Infect. Dis.* Vol. II. 1905. pp. 143–247.

†Various bulletins of the Department of Agriculture. Revenel. *Journal of Medical Research.* X., p. 460. *Ibid.* Transactions of the American Public Health Association. XXIX. Calmette and Guerin. *Annales de l'Institute Pasteur.* III., p. 1024, IV., p. 636.

intestines to the lungs without leaving any trace of their passage. The others hold that where animals are fed with tuberculous material, particles are insufflated in their passage down the esophagus. The fact that cases have been found in which the tonsils were the only seat of tuberculosis in the whole body shows that in such cases at least the infection was from the air passing over the tonsils.

The dissemination of the tubercle bacillus is doubtless very largely due to the prevalent habit of expectorating in public places. Out of fifty-six samples of sputum collected in street cars by Dr. W. G. Bissell, City Bacteriologist in Buffalo, four were tuberculous. In forty-eight samples taken from the floors of the public building by Dr. C. R. Orr, of the pathological laboratory of the University of Buffalo, tubercle bacilli were found three times. According to the researches of Nuttall, a person suffering from tuberculosis may expectorate many millions of tubercle bacilli in the course of twenty-four hours. Coughing and sneezing may serve to disseminate the bacilli (see page 175).

Concerning the occurrence of tubercle bacilli in cow's milk and butter, and in beef, see pages 151, 351 and 152.

Cases have been recorded in which the disease was transmitted from the mother to the child in the uterus; how frequently this happens is uncertain. It is usual to attribute greater importance to an inherited tendency to tuberculosis than to the inheritance of the tubercle bacilli themselves.*

Agglutination of the tubercle bacillus is said to occur with the serum of cases of tuberculosis under certain circumstances. The reaction does not seem likely to be of practical value.

Tuberculin is made by concentrating a culture of tubercle bacilli grown in glycerin-bouillon to one-tenth of its original volume, over a water-bath, and filtering through a sterilized

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*On tuberculosis of the placenta see Warthin. *Journ. Infect. Dis.* Vol. IV. 1907. pp. 347-368.

Pasteur-Chamberland bougie of unglazed porcelain. It therefore represents the products of tubercle bacilli. It was proposed by Koch as a remedy for tuberculosis, but it has not met with great success, and is little used as a therapeutic agent. It has been found, however, of great value in the diagnosis of tuberculosis, especially in cattle. When tuberculin is injected into a tuberculous animal there results considerable general disturbance, of which the most noticeable evidence is a sudden rise in temperature, while hyperemia is excited around the tuberculous area. In a healthy subject the injection produces no reaction; but there is, nevertheless, danger attending its use. To avoid the objections to the injection of tuberculin in human beings, Calmette* and Wolfe-Eisner independently recommended the application of tuberculin to the conjunctiva as a means of diagnosis. A marked hyperæmia of the conjunctiva follows this procedure in tuberculous individuals, while this is very slight or entirely absent in healthy persons. In tuberculous patients aside from some discomfort and interference with vision there is no serious consequence as a rule, though in some cases there is, however, considerable œdema and even purulent exudation. As a diagnostic measure in cattle† it has been found accurate in the great majority of cases. Concerning tuberculosis in cows, see page 151. Supposing that some curative principle exists in the bodies of the tubercle bacilli themselves which could not be procured from cultures deprived of their bacilli by filtration through porcelain, Koch has recently proposed a new form of tuberculin called "tuberculin R," which consists of an extract made from dried and pulverized living tubercle bacilli. The value of this new tuberculin as a remedy is at least doubtful, and physicians are disposed to regard it as dangerous.

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*Calmette. Comptes rendus de l'Académie des Sciences. June 17, 1907.

†For details as to its use in cattle see V. A. Moore. Infectious Diseases of Animals. 1906. p. 196.

Weber* obtained a positive reaction in five healthy physicians who tried the intraocular injection. The explanation in these cases is to be found in the fact that the men experimented upon were in the habit of sitting up reading late by artificial light.

Immunity from tuberculosis has been attained experimentally to a certain degree. In very old cultures the virulence of tubercle bacilli sometimes becomes greatly diminished. Animals which survive injections of such bacilli may afterward withstand large doses of virulent bacilli.†

Friedmann‡ has succeeded in rendering guinea-pigs and also cattle immune to injection with virulent tubercle bacilli by injections of cultures of turtle tubercle bacilli. A single injection of such cultures which are in themselves apparently harmless, confers a high degree of immunity upon cattle.

Acid-proof bacilli resembling tubercle bacilli have been alluded to a number of times (pages 33, 36, 163, 351). A number of such bacilli have been cultivated, such as those of butter and grass. Injected into animals they may produce nodules more or less like tubercles. In these nodules they sometimes assume forms resembling the fungus of actinomycosis. The tubercle bacillus rarely shows similar forms. All the bacilli of this class, including the tubercle bacillus, sometimes show branching. It is probable that the bacilli of this group are related to the fungus of actinomycosis.§ Similar organisms have been found in fishes, in whom they produce nodules resembling tubercles; it is quite possible that the latter organisms are tubercle bacilli, which have been modified by an altered environment. Another acid-proof bacillus has been found which is pathogenic to rats, producing lesions of the skin with nodules; the disease appears in wild rats in certain localities.

Tuberculosis of Birds.—Fowls, ducks and other birds sometimes suffer from tuberculosis due to a bacillus closely resembling the tubercle bacillus of mammals. It has similar staining properties. It sometimes grows in long, branching forms. It differs somewhat from the tubercle bacillus of mammals in its cultural properties. The liver is the organ most often affected. Guinea-pigs are much less susceptible to it than to mammalian tuberculosis. Rabbits are somewhat susceptible, though less so than to mammalian tuberculosis.

Pseudotuberculosis.—Guinea-pigs and other rodents sometimes present lesions macroscopically very similar to those of tuberculosis, in which, however, the tubercle bacilli cannot be found. These affections appear not to be tuberculosis at all, and their nature is not well understood. Several organisms have been found in them, all of which are entirely unlike the tubercle bacillus.

Bacillus Lepræ (bacillus of leprosy).—A slim bacillus about 4 μ in length. It is probably not motile. It is uncertain

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British Med. Journ. Feb. 1908. Cited in *Journ. Amer. Med. Assn.* March 1, 1908.

†Trudeau. *New York Medical Journal.* July 18, 1903. Salmon. *Philadelphia Medical Journal.* June 13, 1903.

‡*Deutsche Méd. Wochenschr.* XXX., No. 46. 1904.

§Abbott and Gildersleeve. *University of Pennsylvania Medical Bulletin.* June, 1902. Borrel. Bacilles Tuberculeux et Paratuberculeux. *Bulletin de l' Institute Pasteur.* T. II., p. 409 and p. 505.

whether or not it forms spores. It stains by the Gram and the Weigert fibrin method but requires to be stained for a longer time than most Gram positive bacteria, and it is also colored by the methods used for staining the tubercle bacillus. It takes the dye, however, more readily than the tubercle bacillus. In stained preparations it appears very similar to the tubercle bacillus, and resembles it in having alternate colored and unstained spots. Babes mentions short branching forms with inverted pear-shaped extremities. Although several observers have reported success in attempts to cultivate the bacillus of leprosy, their claims have been disputed. Organisms resembling the diphtheria bacillus, two different kinds have been cultivated on artificial media by Babes* and others, but Babes regards these as merely adventitious.

The leprosy bacilli lie for the most part deep in the skin, but Babes also found them in the hair-follicles. He also found them in the sputum, nasal secretion, in the conjunctival sac, in the sperma, urethral secretion and elsewhere, but not in the urine; only in small numbers in the mucous membrane of the bladder, twice in the milk, once in the feces, in small numbers in the pleura and peritoneal fluid. They are usually absent from the blood, found only once in 12 cases. Often in the vaginal secretion in those affected. They are very abundant in the ulcers.

The results of inoculation into man and the lower animals of material coming from cases of leprosy have been uncertain. The bacillus of leprosy has been found so constantly in the tissues of those having the disease that it is generally admitted to be the specific cause. The skin and the peripheral nerves are the parts most affected, although other tissues and the internal viscera may be involved. A granulation tissue, forming nodules and thickenings, appears in the affected parts. The bacilli are found in large numbers in the nodules, partly outside of the cells, but mostly within the cells. Most persons who are affected with leprosy have the bacilli in the nasal secretion.* The disease must be communicated directly from one individual to another, for no explanation can be given for the appearance of the infection in any patient, except by communication with some other case. Still transmission by contact seems not to take place easily.

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*Kolle and Wassermann. Ergänzungsband. 1906. p. 160.

Bacillus Mallei (bacillus of glanders).—A slim bacillus with round or pointed ends, which often shows alternate light and dark spots in stained preparations. Branching forms have been described. It is not motile. It probably does not form spores. It does not retain the stain by Gram's method. After staining with the ordinary aniline dyes it is easily decolorized, and on that account it is difficult to demonstrate in sections of tissues. It is facultative anaerobic. It grows at the room temperature, but better in the incubator. It grows slowly on gelatin, and does not liquefy it, or only after a long time. On agar it produces a moist, white growth; on blood-serum, a yellowish or brownish growth; blood-serum is not liquefied. Milk is coagulated slowly, and the reaction becomes acid. On potato, the growth one or two days in the incubator is translucent amber-yellow, later a reddish brown, while the surface of the potato becomes discolored.

In the first few days on potato, cultures resemble those of B. pyocyaneus on this medium.†

In the horse and ass it produces the disease known as glanders, which affects the mucous membrane of the nasal cavity. When the skin is involved, the disease goes by the name of farcy. In the nose, nodules appear in the mucous membrane which become necrotic, forming ulcers. They may become confluent, and may extend along the adjacent surfaces as far as the lungs. There is a profuse discharge

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*Günther. *Loc. cit.* p. 509.

†Frothingham. *Journal of Medical Research.* Vol. VI., p. 334.

from the nose. The neighboring lymph-nodes become involved and are swollen, and nodules may be present in the internal viscera. In the skin the nodes lying underneath the skin are called farcy-buds. Histologically the nodules consist of a granulation tissue, but they tend to break down rapidly, and the process in some respects is very like ordinary suppuration.

In addition to the spontaneous infection of horses and asses, cultures are pathogenic* for guinea-pigs, European field-mice and cats; rabbits, sheep and dogs are less susceptible or only slightly so; also white and house-mice, and hogs; cattle are immune. Camels are susceptible, hedgehogs also. Men are occasionally infected, especially those who come much in contact with horses. The mucous membranes of the nasal cavity may be the part involved, or the skin or the internal viscera. In a number of instances, workers in the laboratory have been accidentally infected.

The diagnosis of the disease is best effected by the inoculation of a male guinea-pig with the material from a case suspected of being glanders, introducing it into the peritoneal cavity (method of Straus). Frothingham† describes the method of procedure which he employs as follows:

The material from the suspected animal—nasal secretion or skin lesion or both—is obtained by using a swab such as is used to obtain material for diagnosis in diphtheria. After it has been applied to the lesion the cotton plegget is removed to a convenient quantity—2 or 3 c.c.—of sterile water, and agitated. The water is then injected in equal portions into the peritoneal cavity of two guinea-pigs. In about two to three days after an inoculation of this kind there appears a characteristic swelling of the testicle, indicating the beginning of suppuration, which presently takes place; the animal usually dies after two or more weeks. At least two guinea-pigs should be inoculated; and the test may sometimes fail, when it should be repeated on other guinea-pigs.* The test may also fail on account of the death of the guinea-pigs from peritonitis.

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*The statements of different writers differ considerably with regard to some of these animals.

†*Journ. Med. Research.* Vol. VI. 1901. pp. 331–340.

Frothingham further points out that there is another organism beside B. mallei which produces ulceration of the guinea-pig testicles which may, however, be distinguished from the latter on potato cultures by its white growth. The brownish-yellow growth of B. mallei on potato resembles the growth of B. pyocyaneus at first, but the latter causes in two or three days a greenish discoloration of the potato. B. mallei does not do this.

Mallein is a product obtained from old glycerin-bouillon cultures of Bacillus mallei. The cultures are sterilized in a steam sterilizer at 100° C. for several hours, and are filtered through a Pasteur-Chamberlin cylinder of unglazed porcelain. The filtrate contains the products of the growth of the Bacillus mallei and is of much the same character as tuberculin. Injected into animals suspected of having glanders, if it produces a local and febrile reaction, the existence of glanders is indicated. This reaction is of use in the diagnosis of the disease in lower animals, especially in horses, where it has been largely employed, though it sometimes fails. An agglutination reaction has been described for the bacillus of glanders.

In regard to this reaction Moore and Taylor† find that it is easier to perform and quite as accurate as the mallein test.

Actinomyces Bovis‡ (Streptothrix actinomyces; Ray-fungus of Actinomycosis).—The morphology of this organism is quite different from that of most of the bacteria. It is sometimes considered to be a bacterium of a higher type. The organism appears in the form of threads which show genuine

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*Frothingham. *Journal Medical Research.* Vol. VI. 1901.

†*Journ. Infect. Dis.* Supplement No. 3. 1907. pp. 85-94.

‡Hektoen. *Philadelphia Monthly Medical Journal.* November, 1899. Ewing. *Bulletin Johns Hopkins Hospital.* November, 1902.

branching. These threads make radiating, interlacing masses. Their external ends are swollen and bulbous under certain conditions. Colonies formed in this manner, seen under moderate magnification, have a radiating appearance which has given rise to the name, ray-fungus. The club-shaped external ends are readily distinguished and the growth possesses a very distinctive form. This is the shape which the organism presents as it grows in the animal body. The club-shaped ends are generally regarded as a degenerative or involution form. Transverse divisions may sometimes be distinguished upon the threads. Spherical forms resembling micrococci may appear which may possibly be spores. In some members of this group spores—conidia—form in cultures on the ends of the filaments. While the organism stains with the ordinary aniline dyes, by Gram's method or the Weigert fibrin stain. It is absolutely essential that the anilin oil-gentian violet be prepared with a strictly saturated alcoholic solution of gentian violet, and that the stain should be a few days old, not freshly prepared.*

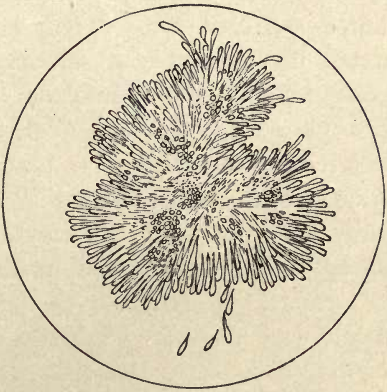

FIG. 93.—Ray-fungus of actinomycosis. Fresh, unstained preparation from a case of lump-jaw in a cow. (*Diagrammatic.*)

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*Günther. *Loc. cit.* p. 780.

The fungus may be cultivated upon the usual culture-media, though not easily. It is facultative anaerobic. It grows both at ordinary temperatures and in the incubator. The growth is not rapid. The colonies are fine, dry, elevated, irregular in form, becoming opaque. Bulbous ends upon the threads do not usually appear in cultures. The results of the injection of these cultures into the lower animals are as yet uncertain. Most authors report failure to obtain positive results of any kind and no one has yet succeeded in producing typical actinomycosis by inoculating pure cultures.*

The disease produced by the ray-fungus is called actinomycosis. It occurs in cattle chiefly, seldom in swine and horses, and occasionally in man. Infection appears to be carried by grain or particles of vegetable fiber which penetrate the tissue. The presence of such foreign particles as well as the organisms appears to favor infection. The infectious material frequently enters through the mouth, especially in the vicinity of the teeth, but it may also occur through the skin or the mucous membranes. It leads to the formation of inflammatory, tumor-like nodules, hence the name "lump-jaw" given to the disease in cattle. Necrosis of the tissue takes place with the formation of an abscess. The pus is peculiar in containing small yellowish-white particles—so-called "sulphur granules"—which consist of little clumps of the ray-fungus, and which readily permit the disease to be diagnosed by the microscope. The material may be examined in the perfectly fresh condition without any staining. The jaw or its neighborhood is very frequently affected, or the disease may be present in other situations about the head and neck, and may involve the lungs, the intestines and the vertebræ, ribs and other bones. The disease is usually localized, but a number of areas may be affected simultaneously.

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*Kolle and Wassermann. *Loc. cit.* p. 879.

As a result of his research, Wright* comes to different conclusions from many of those who have studied the ray-fungus. He regards the organism as essentially anaerobic; seldom found in external nature; probably present in the normal alimentary canal. He succeeded in obtaining club shapes in serum cultures, and in producing nodules by inoculation of animals. The bodies called spores by other observers are not to be regarded as spores.

According to Wright's description, the organism represented by Fig. 89 would have to be regarded not as the true ray-fungus but as one of its saprophytic congeners.

Besides the common actinomyces, there are numerous other ray-fungi, more or less closely related, and whose pathogenic properties are not fully determined. Generally speaking, they appear to be essentially saprophytes, which occasionally become parasitic and pathogenic under especially favorable conditions. A number of species have been found in air, dust, etc., some of them chromogenic. Wolff and Israel described an anaerobic species, pathogenic to man and animals. Madura disease, Madura foot, or mycetoma is a disease occurring in India (rarely elsewhere), affecting one of the extremities, characterized by swellings, nodular deposits and abscesses. Some cases are certainly due to a member of the aetinomyces group.†

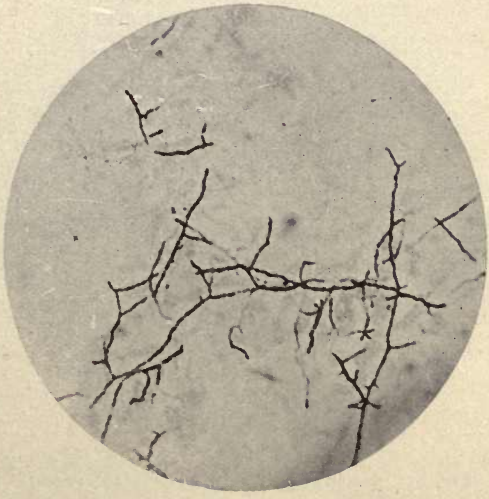

FIG. 94.—Actinomyces bovis, smear preparation from a pure culture, stained by Gram's method. (X 1000.)

Other branching organisms, some of them acid-poof, have been described

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*Wright. *Journal of Medical Research.* XIII., p. 349.

†Compare Wright. *Journal Experimental Medicine.* Vol. III., p. 421.

chiefly under the name of streptothrix. In man they have been found in a variety of suppurative and necrotic lesions, in particular, bronchopneumonias.*

Bacillus Typhosus (Bacillus of Eberth).—A bacillus with rounded ends, varying in length, sometimes making very short, oval forms, sometimes growing out into long threads. It is very actively motile, and possesses numerous flagella which arise from all parts of the surface. It does not form spores. It is not stained by Gram's method, but it may be colored with the ordinary aniline dyes, when the stain will frequently be somewhat irregular. It may be stained in sections of tissues from cases of typhoid fever, with the aniline dyes, such as Löffler's alkaline methylene-blue. It is a facultative anaerobe. It grows at ordinary temperatures, better in the incubator, but grows rather more slowly than B. coli communis. Gelatin is not liquefied. Young surface colonies in gelatin appear

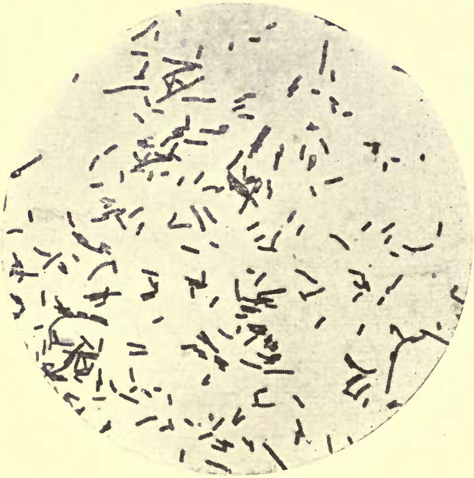

FIG. 95.—Bacillus of typhoid fever. (× 1000.)

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*Norris and Larkin. *Journal Experimental Medicine.* Vol. V., p. 155. Musser. *Philadelphia Medical Journal.* September 7, 1901. Flexner. *Journal Experimental Medicine.* Vol. III. MacCallum. *Centralblatt für Bakteriologie.* Orginal. Bd. XXXI. 1902.

whitish, with irregular borders and more or less wrinkled surfaces, when slightly magnified. It grows on the ordinary media, and the growths are whitish. Bouillon is clouded. Milk becomes slightly acid, but is not coagulated. In media containing dextrose, acid is formed but no gas. In lactose-bouillon neither acid nor gas is formed, although when grown in milk the typhoid bacilli produce an acid reaction; but this acidity is not due to a fermentation of the milk-sugar, but to a substance resembling glucose as was pointed out by Theobald Smith.

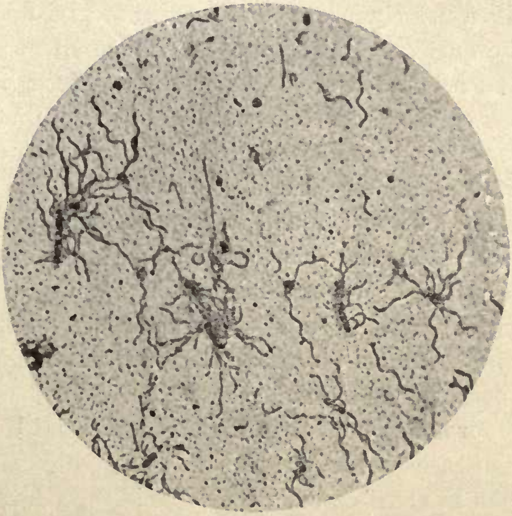

FIG. 96.—Bacillus of typhoid fever, stained by Löffler's method to show flagella. (× 1000.)

In Dunham's peptone solution indol is not formed in one or two days, but in cultures grown for 10 days at 37° C. there is always indol formed,* as a rule.

On the lactose-litmus-gelatin or agar of Würtz the blue tinge possessed by colonies of the typhoid bacillus on this medium is made use of to distinguish

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*Chantemesse, Morris (Quoted from Günther *Loc. cit.* p. 526).

them from colonies of the colon bacillus and other bacteria which form acids from lactose. Neutral-red has been used in the same manner, as it is not altered by the typhoid bacillus, but is changed by the colon bacillus to a yellow color. This medium is prepared by adding to neutral, plain agar .05 grams of neutral-red to 1 liter of agar or 1 per cent. of a saturated aqueous solution of neutral red, some also add 0.3 per cent. dextrose. The material to be examined is shaken up with the melted agar and the tubes placed upright in the incubator at 35–37° C. These constitute the "shake tubes" mentioned in books on water examination. Neutral-red may also be used in the same proportions as an addition to beef-broth, and the cultures made in fermentation tubes.

On potato it usually forms what is called an invisible growth; that is, although no development is apparent to the eye, numerous bacilli may be shown under the microscope in smear preparations made from the surface of potato inoculated about forty-eight hours previously. Occasionally a slight visible growth is seen on potato.

The typhoid bacillus is killed at 60° C. in ten minutes moist heat; though Clark and Gage found that individual bacilli may withstand 80° C. in fluid cultures for 5 minutes. It resists drying well. It can survive in soil and sewage a long time.

For a comparison of the properties of the typhoid bacillus and the colon bacillus see the latter.

There it probably no other organism associated with an infectious disease which presents so much difficulty in its identification in given cases as the typhoid fever bacillus, and yet there is none which so frequently demands early and prompt identification. Bacteriologists are constantly confronted with the demand from communities, physicians, and laymen to give a positive answer as to whether a specimen of water, milk, feces or other material does or does not contain the typhoid bacillus. This demand has led to many efforts on the part of bacteriologists to work out special methods for the isolation and identification of the organism. These efforts have placed in the hands of those who have had long training the means of saying with some probability, but hardly yet with absolute certainty, in all cases at least, that the typhoid bacillus is or is not present in the material examined.

The crucial test of animal inoculation which furnishes in the case of anthrax, tuberculosis, glanders and other diseases such a sure means of diagnosis cannot be resorted to in the case of typhoid fever for the reason that experiment animals are not subject to spontaneous typhoid and do not take the disease on inoculation with cultures of the typhoid bacillus. It is true that inoculation of animals with the cultures is followed by disease, and the experiments at one time rather encouraged the hope that the results obtained by the inoculation of animals with cultures of the typhoid bacillus would furnish a trustworthy means of diagnosis. But the symptoms and lesions produced by the inoculation of typhoid bacilli are so similar to those produced by the inoculation with certain other bacteria that such inoculations cannot be depended upon for differentiation. Some strains of the very bacteria, those of the colon group, which it is important to separate from the typhoid bacillus are so similar to the typhoid in pathogenic properties for experiment animals that a diagnosis between them on this ground is not possible, and it becomes necessary to resort to cultural characteristics and to certain other more or less unsatisfactory tests.

Of the various media which have been devised, and the various tests which have been recommended the following may be described.

Löffler introduced a medium consisting of agar-agar colored with malachite green. All bacteria including the typhoid bacillus are retarded in growth upon this medium, and the typhoid bacillus along with certain others change the color of the medium to yellow.*

The medium suggested by Hiss† for the isolation of the typhoid bacillus consists of gelatin and agar, beef-extract, sodium chloride and dextrose, and is given a slightly acid reaction. These substances are used in different proportions for plate- and for tube-cultures. This medium is of a semi-solid character,

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*Günther. *Loc. cit.* p. 534.

†*Journal Medical Research.* Vol. VIII. 1902.

and the great motility of the typhoid bacillus produces a uniform clouding of the medium in tubes, without gas-formation, characters which distinguish this organism from the colon bacillus; in plate-cultures with this medium the colonies exhibit peculiar filamentous outgrowths. It is claimed that it can be determined whether organisms are typhoid bacilli or not after thirty-six hours in the incubator by this method.

Other special media for the identification of the typhoid bacillus have been devised by Elsner, Stoddart, by Capaldi and Proskauer, and by Piorkowski.* The medium of Stoddart is based upon principles similar to those applied in the medium of Hiss.

The Drigalsky-Conradi† method for isolating the B. typhosus from water and feces is that now most employed. The principle of this method consists in the use of a culture-medium on which the surface colonies of B. coli and of B. typhosus each show a characteristic, macroscopic appearance, so that they can be separated from one another. Furthermore, the medium employed is unfavorable to the growth of many bacteria likely to be present.

The addition of crystal violet to the milk-sugar-litmus-agar inhibits the growth of various organisms without materially affecting the growth of B. typhosus. The medium adopted by Drigalsky and Conradi after many trials was as follows:

(*a*) Three pounds of chopped beef placed over night in 2 liters of water. Strain off, and boil for one hour, filter and add 20 grams of Witte's peptone, 20 grams of nutrose, and 10 grams of salt. Boil one hour, filter and add to it 60 grams of best stick agar; boil three hours over the flame or one hour in the autoclave, make slightly alkaline to litmus paper, and boil one-half hour.

(*b*) 260 c.c. litmus solution (Kubel and Tiemann); boil for ten minutes; add 30 grams of c. p. milk-sugar, and boil the mixture fifteen minutes.

(*c*) Add solution *b* to the hot, melted solution *a*; mix thoroughly and correct the reaction to weakly alkaline if not already so.

(*d*) Add 4 c.c. of a hot, sterile 10 per cent. solution of dehydrated soda.

(*e*) Add 20 c.c. freshly prepared $\frac{1}{10}$ per cent. solution of crystal violet (Krystallviolet "B," Höchst). This solution should be made with warm, sterilized, distilled water, but not boiled.

A part of this agar is poured into Petri dishes at once. The rest is kept in flasks, about 200 c.c. in each.

The material to be examined is spread over the surface of the plates, not mixed with the medium as is usually done, the object being to obtain surface colonies only.

The spreading is done by means of a glass rod 12 or 14 cm. long, bent at right angles about 3 cm. from one end. The short arm of the bend terminates in a small knob, and is dipped into the material to be examined and run over the surface of the agar in a series of the previously prepared Petri dishes.

Drigalsky-Conradi plates, as described above, are made from water by using the precipitate after centrifuging.

Ficker and Hoffmann‡ recommend the following method of treating the

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*Elsner. *Zeitschrift für Hygiene.* Bd. XXI., p. 25. 1895. Stoddart. *Journal of Pathology and Bacteriology.* Vol. IV., p. 429. 1897. Capaldi and Proskauer. *Zeitschrift für Hygiene*, etc. Bd. XXIII., p. 452. 1896. Piorkowski. *Berliner klinische Wochenschrift.* p. 145. 1899.

†Drigalsky-Conradi. Ueber ein Verfahren zum Nachweis der Typhusbacillen. *Zeitschrift für Hygiene und Infectionskrankheiten.* Bd. XXXIX. 1902.

‡Ficker and Hoffmann. Weiteres über den Nachweis von Typhusbacillen. *Archiv für Hygiene.* Bd. XLIX. 1904.

material for examination for the typhoid bacillus before making the Drigalsky-Conradi plates. They use an enriching fluid which has the property of inhibiting the growth of colon and other contaminating organisms, while not seriously interfering with the growth of the typhoid bacillus.

This fluid consists of:

(*a*) A stock solution of beef-broth. Take one kilogram of chopped beef; add 2 liters of distilled water; heat thirty minutes at 50° to 60° C.; stir; boil for thirty minutes; fill up water lost by evaporation; press through gauze; measure; add 6 per cent. peptone (Witte); ½ per cent. salt; heat till peptone dissolves; filter; distribute into sterilized beer bottles with patent stoppers; cover with paper cones; sterilize two hours; clamp the stoppers to, and store away.

(*b*) Enriching fluid: 100 c.c. of the above stock solution measured in a sterilized measuring glass; put in a sterilized Erlenmeyer flask; add sodium hydrate solution, 2.7 c.c. less than the quantity required for phenolphthalein "red point," as determined by neutralizing 25 c.c. Sterilize ten minutes—in steam; allow to get cool; add 105 c.c. of a 1.2 per cent. solution of caffeine (solution to be made fresh in cold, sterilized, distilled water every time). Add 1.4 c.c. of a $\frac{1}{10}$ per cent. solution of crystal violet; crystal violet must be dissolved cold.

(*c*) Preparation of the stool:

1. Thin stool allowed to settle, eight-tenths or nine-tenths c.c. of the thin, upper portion added to *b*.

2. Semifluid stool rubbed up in a mortar with 1 part of 1.2 per cent. solution of caffeine; filter through sterilized cotton-wool; eight-tenths or nine-tenths c.c. of the filtrate added to *b*.

3. Thick feces. Rub 1 part of feces with 2 parts of caffeine solution, and proceed as in No. 2.

In all three cases shake thoroughly and place at 37° C.

(*d*) Search for typhoid fever bacillus. Examine a hanging-drop.

1. If there are relatively few bacteria, make 6 large Drigalsky agar plates: plate No. 1 of the series with 0.30 to 0.35 c.c. of the fluid; plate No. 3 with 0.25 c.c.; plate No. 5 with 0.10 c.c. Plates Nos. 2, 4 and 6 are the diluted plates from Nos. 1, 3 and 5.

2. If there is abundant growth, 7 plates are made: 1, 4 and 6 are inoculated with 0.2 c.c., 0.15 c.c. and 0.1 c.c., respectively, and the others are dilutions from these.

Identification of colonies as usual.

Keep the rest of the culture in the enriching fluid on ice. If the first plates fail for any reason, shake this enriching fluid with glass beads and make Drigalsky plates again.

A simpler way of preparing the Drigalsky-Conradi medium is recommended by Hagemann* as follows:

Liebig's extract, 10 grams; Witte's peptone, 10 grams; sodium chloride, 10 grams; water, 600 c.c. Boil in a salt-water bath until 100 c.c. evaporates off. Add 500 c.c. fresh, raw, amphoteric milk. Boil and add agar, 10 grams. Boil until the agar is nearly dissolved; put in the autoclave for twenty to thirty minutes at 110° to 115° C. Filter in the streaming steam. Divide up into sterile Erlenmeyers, about 200 c.c. in each. Sterilize a short time.

In using, melt in a water-bath; add normal sodium hydrate till the reaction is slightly alkaline to litmus-paper. Add 20 c.c. Merck's litmus solution. Also add 3 drops of a 1 per cent. alcoholic solution of crystal violet. Mix thoroughly. Pour into Petri dishes, and use as in the original method.

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*Hagemann. Eine Vereinfachung des Drigalskischen Nährbodens. *Hygienische Rundschau.* Vol. XIV. 1904.

M. W. Richardson has devised an application of the serum-test to plate-colonies suspected of containing typhoid bacilli. If a typhoid colony be torn with a needle, under moderate magnification "a seething motion resembling much the appearance of a swarm of bees" may be seen. This appearance is due to the motility of the bacteria. If such a colony be touched with a small quantity of blood-serum from a case of typhoid fever, the motion is said to cease instantly and almost absolutely. Colonies of other motile bacteria do not undergo a corresponding loss of motility.

The Serum-test for Typhoid Fever.*

When a small quantity of a culture of typhoid bacilli is mixed with a little blood-serum derived from a case of typhoid fever, within a few minutes the motility of the typhoid bacilli ceases and they become agglutinated into clumps or masses (see Agglutinins, Bacterial Poisons, page 190). The bacilli may eventually undergo disintegration into granular material (see Lysins, Bacterial Poisons, page 193). This reaction rarely takes place with the blood-serum of healthy persons or of those suffering with other diseases, nor when the blood-serum of a typhoid fever case is mixed with motile bacteria other than typhoid bacilli. It has been observed in the blood-serum of an infant born while the mother was convalescing from typhoid fever.

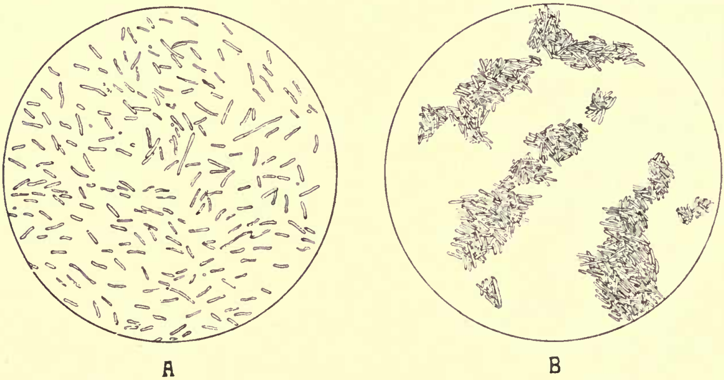

Fig. 97.—Application of the serum-reaction to typhoid bacilli. A shows the distribution of the bacilli before the reaction. It is to be remembered that they are motile and their positions may change continually. B shows clumping of the motionless bacilli after mixture with the serum of a case of typhoid fever. (*Diagrammatic.*)

The agglutinating substance has been found in blister-serum and in the milk of typhoid cases, in fluids from the serous cavities and inflammatory and edematous areas in variable amounts, and occasionally in urine, bile and tears.

The reaction may be obtained by adding blood-serum to a young bouillon-culture of typhoid bacilli kept in the incubator, when the occurrence of agglutina-

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*This test is often known as the "Widal reaction." For a history and general discussion of the subject see Durham. *Journal of Experimental Medicine.* Vol. V. p. 353.

tion becomes manifest by the collection of the bacteria into visible masses or flocculi, which form a sediment. Most investigators prefer to watch the results under the microscope, using an ordinary slide, or, better, the hanging-drop. Young cultures—less than twenty-four hours old—in bouillon, and kept in the incubator, may be used, or, better, cultures kept at room-temperature for twenty-four hours. Johnston and McTaggart recommend that the bouillon cultures be freshly made each time from stock cultures on agar, which need only occasionally be transplanted. Certain stocks of typhoid bacilli seem especially suited to this reaction, and such a stock should be secured.

Blood-serum, blister-serum, fresh blood and dried blood have all been used with success. Blood dried on unglazed paper or cover-glasses as proposed by Wyatt Johnston is extremely convenient. To perform the test the drop of dried blood is mixed on the paper with sterilized bouillon or normal salt solution, and portions of the suspension of the blood-serum obtained in this way are tested upon the typhoid cultures. The objection to this procedure lies in the dificulty of securing an accurate dilution. An approximate knowledge of the degree of dilution may be acquired by mixing drops of dried blood of known volume with definite amounts of water, and observing the tints. These should be kept in mind as standards. The dilution may be measured with the hemoglobinometer or with the pipette of the hemocytometer. The New York Board of Health have found blister-serum satisfactory and easy to obtain. A little of the diluted serum is mixed on the cover-glass with a definite amount of the fresh bouillon-culture, and is examined as a hanging-drop. In a short time the characteristic clumping and loss of motility occur. At the same time a drop of the culture alone, and a drop of the culture mixed with *normal serum*, similarly diluted, should be examined as controls. The dilutions finally resulting after mixing with the drop of culture, when placed under the microscope, vary from one part of serum in 30 to 1 in 50. The higher dilution at which clumping takes place the more definite the result. The time within which the reaction occurs varies from a few minutes to about one. With little dilution the time should be short; with greater dilution it may be longer. Both cessation of motility and clumping should take place. In a positive case the cessation of motion and the clumping of the bacilli should be complete. Normal blood sometimes exhibits agglutinative properties in some degree. Some cultures show a tendency to clump without the addition of any blood serum, but such sources of error are checked by the control tests described. If the reaction in any case is not satisfactory, it should be tried with a higher dilution, 1 to 50 or more, and the result should be positive if the case is a genuine case of typhoid fever.

The agglutinating power usually appears in the blood between the seventh day and the end of the third week of the disease; it may be seen earlier; it is often delayed and appears late. The test frequently has to be repeated when the first result is doubtful or negative. Reports indicate that the method is a great aid in the diagnosis of typhoid fever, though not infallible.

The use of dead cultures for the agglutination reaction has been recommended by various authors, and Ficker has placed his so-called "Diagnosticum," a suspension of dead typhoid bacilli, on the market. For this method of diagnosis Ruediger* makes the following recommendation: Inoculate a large flask—100 or 1,000 c.c.—with the typhoid bacillus and incubate at 36° C. for 24 hours. At the end of this time add 1 c.c. of formalin for every 100 c.c. of broth. This is now ready for use, and can be preserved for many months. It must be shaken up every time before use to distribute the organisms. Ruediger further advises that in making the test four drops of blood be mixed with 2 c.c. of a 2 per cent. for-

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**Journ. Infect. Dis.* Vol. I. 1904. pp. 236–240.

malin solution, and that 1 c.c. of this diluted blood be added to 4 c.c. of the dead culture. This mixture is set aside along with a control tube made with normal blood or with distilled water. If agglutination takes place, the dead organisms settle down in a flocculent precipitate in an hour or two.

By the use of a medium consisting of 90 c.c. of ox-bile, 10 c.c. of glycerin, and 2 grams of peptone. Coleman and Buxton* have come to the conclusion that the typhoid bacillus is present in the blood in every case of typhoid fever throughout its course. These investigators distribute this medium into small flasks, 20 c.c. into each, which are then sterilized. The blood to be examined is drawn by means of an all glass syringe from the vein at the bend of the elbow, and 3 c.c. of it introduced into each of three of the flasks of ox-bile medium. After incubation over night, streak cultures are made from the surface of the liquid in the flasks on the surface of litmus-lactose-agar plates. Further procedures for the identification of the organism are those already given.

Considerable experience is necessary to acquire the judgment needed in using this test.

The agglutinating power becomes lessened after recovery, and usually is wanting at the end of a year. Rarely it may be present for a longer time, a fact that is to be borne in mind in making a diagnosis.

Various observers have obtained the Widal reaction with serum patients suffering from other diseases than typhoid.

Typhoid bacilli have frequently been obtained from the stools of cases of the disease, but they are isolated only with considerable difficulty. At autopsies they are best cultivated from the spleen, in which, however, it is to be remembered, the Bacillus coli communis may also be present. Cultures made from the blood, where several cubic centimeters are taken, show that a few bacilli occur in the blood in a large proportion of cases of the disease—probably in a majority. Typhoid bacilli appear in the urine in about 20 per cent. of all cases, and the examination of urine for them has been used in diagnosis. The bacilli often occur in the gall-bladder. They, as well as the colon bacillus, have been found inside of gall-stones, and have been supposed to be one of the causes for the formation of gall-stones.† They may remain present in the gall-bladder or in the urine‡ long after convalescence from the disease. They have been demonstrated in the "rose spots" on the abdomen. They may be present in the lesions

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*Reprint *Am. Jour. Med. Sci.* June, 1907.

†Pratt. *American Journal Medical Sciences.* Vol. CXXII. 1901. Also Kramer. *Journ. Exper. Med.* Vol. IX. 1907. pp. 319–323.

‡M. W. Richardson. *Journal Experimental Medicine.* Vol. IV. 1899.

of the pneumonia,* which frequently complicates typhoid fever, and may appear in the sputum. In times of epidemic of the disease they may be present in the stools of persons who show no symptoms of typhoid fever. Nieter† found 13 such typhoid fever bacillus carriers in a certain German insane asylum.

Inoculation experiments in animals have not been very satisfactory. With a few possible exceptions, anatomical lesions resembling those of typhoid fever have not been produced by the inoculation of typhoid bacilli into animals. The injection of cultures into animals may produce death, but it can usually be shown to have resulted from the poisons contained in the cultures.

Besredka‡ obtained endotoxins from the bacilli of typhoid fever, plague and dysentery by triturating with salt to an impalpable powder, adding water, drop by drop, allowing to remain over night, and with the typhoid bacilli heating at 60 to 62° C. for two hours, allowing to settle and decanting the liquid which contains the endotoxin with the plague and dysentery bacilli the separation is affected without heat by centrifugalizing.

Typhoid fever is rare during the first two years of life. It frequently attacks young and robust men. The causes that bring about susceptibility to infection are not known.

The principal lesion in typhoid fever lies in the Peyer's patches of the lower part of the small intestines; the mesenteric lymph-nodes and spleen also are swollen. The typhoid bacillus may be demonstrated in sections of the walls of the diseased portion of the intestines. Cases are recorded in which no lesions were found in the intestines, but where the typhoid bacilli were widely spread through the organs of the body, and which therefore represented typhoid septicemia.

Periostitis and osteomyelitis, which are not uncommon sequelæ of typhoid fever, may be caused by typhoid bacilli.

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*Robinson. *Journ. Infect. Dis.* Vol. II. 1905. p. 498.
†Münchner. *Med. Wochenschr.* Aug. 13, 1907. LIV. No. 33.
‡*Bull. de l'Inst. Past.* Vol. IV., No. 13. July 15, 1906. pp. 586–587.

Ordinary suppuration may be produced by the typhoid bacillus but most suppurative affections during or following typhoid fever are mixed infections, or are due to the ordinary pyogenic bacteria.

That typhoid fever is transmitted chiefly through the medium of water, has long been held, though there are some who now regard other modes of transmission of equal or more importance.

In Washington, D. C., there was a marked increase in the number of cases of typhoid fever after the introduction of the water filtration plant.* After the introduction of the filter plant the purity of the water as shown by the number of bacteria was in inverse proportion to the death-rate from typhoid fever.† Rosenau‡ attributes the typhoid fever in the District to three causes: To importation, to infected milk, and to contact. He attributed 11 per cent. of cases during 1906 and 9 per cent. during the summer of 1907 to milk; 7 per cent. during 1906, 19 per cent. during the summer of 1907 to contact; 15 per cent. during 1906, 25 per cent. during the summer of 1907 to importation. It is sometimes conveyed by milk, by green vegetables and by oysters. Infection through the medium of dust and by the hands and clothing probably occurs, but not commonly. Under certain circumstances the bacilli may be carried by flies,§ and it is consequently of the greatest importance in preventing the spread of the disease to prevent flies from having access to the excreta of typhoid fever patients. In caring for cases of typhoid fever the stools, urine, sputum and linen should be disinfected. Persons handling the patient should wash and disinfect their hands after every contact with the patient.

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*Ed. *Journ. Am. Med. Assn.* Vol. XLVIII. No 17. April 27, 1907. p. 135.

†Cosby. *Wash. Med. Ann.* Report of meetings of the Medical Society, Washington, D. C., Feb. 19, and 26, 1908. p. 135.

‡*Ibid.*

§Vaughan. *Philadelphia Medical Journal.* June 9, 1900.

While the idea formerly entertained that sewer gas is itself the cause of typhoid fever, Horrocks* points out that the bubbles of gas in sewage may be the means of spreading the contagium.

The injection of typhoid bacilli which have been killed by heat has been resorted to as a preventive measure in a large number of cases in the British army. The results appear to have been partially successful, but the method is still in an experimental stage.

Richardson† sums up his results with the use of specific sera, filtrates from cultures, and non-toxic extracts from cultures (Vaughan) approximately as follows: Seventy-four cases were treated by various methods with serum prepared by inoculating horses with typhoid cultures as in the method of producing diphtheria antitoxin; 35 cases were treated with the filtrates through porcelain filters of cultures of typhoid bacilli; 21 cases were treated with non-toxic extracts of the typhoid bacillus prepared by Vaughan. All these methods increase the tendency to relapse unless the Vaughan non-toxic treatment is kept up into the stage of convalescence. The use of serum has no advantages over the filtrates and extracts upon the course of the disease. Filtrates may produce chills, rise of temperature and pulse, followed by a general improvement in the clinical aspect. The non-toxic residue of Vaughan seems to make the typhoid process longer, but milder, and to prevent relapse in convalescence.

Bacillus coli communis (often called simply the *colon bacillus*, Bacterium coli commune of Escherich, and Bacillus pyogenes fœtidus of Passet, who obtained it from foul pus; probably the same as Bacillus Neapolitanus of Emmerich).—A bacillus with rounded ends, frequently of a short, oval form, when it may be difficult to distinguish from micrococci; often longer, even forming threads. It is slightly motile, having several flagella. It does not form spores. It stains with the ordinary aniline dyes, but not by Gram's method. It is a facultative anaerobe. It grows well at the room-temperature, but more rapidly in the incubator. It does not liquefy gelatin. In gelatin plates the surface colonies are of a bluish-white color; the centers are denser than the borders, which are translucent. It usually grows more rapidly in gelatin than the bacillus of typhoid fever. Its growths in other media are mostly whitish. Bouillon becomes clouded. Nitrates are reduced to nitrites.

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*Ed. in *Journ. Am. Med. Assn.* Vol. XLVIII., June 1, 1907. p. 1869.

†Annual Meeting of the Massachusetts Medical Society. Boston. June 11, 1907.

In peptone solution it forms indol. On potato it forms an abundant visible growth from cream color to pale brown. Milk becomes acid and is usually, but not always, coagulated slowly. It causes the development of gas and acid in media containing dextrose or lactose. In media containing neutral red the colon bacillus produces a yellow color with a green fluorescence. Differential points between the bacillus of typhoid fever and the Bacillus coli communis are as follows:

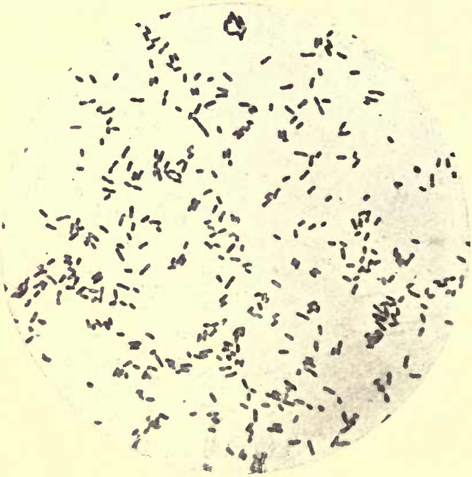

FIG. 98.—Bacillus coli communis. (× 1000.)

1st. The typhoid bacillus is actively motile; the colon bacillus less actively motile.

2d. The typhoid bacillus has numerous flagella which rise from all parts of the surface; the colon bacillus has a smaller number of flagella.

3d. The colonies of the typhoid bacillus in gelatin develop more slowly than those of the colon bacillus.

4th. The superficial colonies of the typhoid bacillus on gelatin, plates are less dense than those of the colon bacillus.

5th. In media containing dextrose or lactose the typhoid bacillus does not produce fermentation with gas and the colon bacillus does produce gas in such media.

6th. The typhoid bacillus produces a very slight acid reaction without coagulation in milk, and the colon bacillus produces a strong acid reaction with coagulation.

7th. In peptone solution the typhoid bacillus, as a rule, produces no indol, and the colon bacillus produces indol.

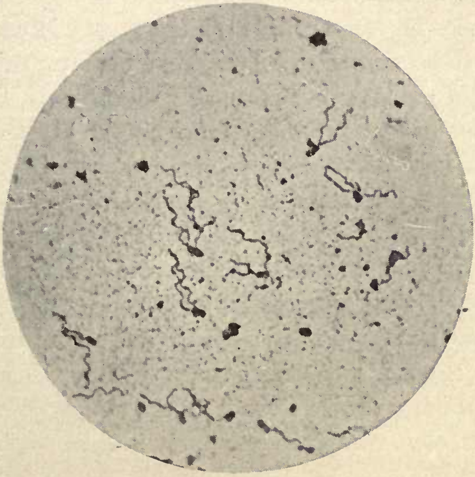

FIG. 99.—Bacillus coli communis with flagella, stained by Van Ermengem's method. (× 1000.)

8th. The typhoid bacillus usually produces an invisible growth on potato, the colon bacillus a visible growth.

9th. The typhoid bacillus is said not to reduce neutral red in media, and the colon bacillus to change it to a yellow color.

To these may be added the growth of the two organisms on special media like those of Würtz, of Elsner, of Hiss and of Drigalsky and Conradi and the application of the serum-reaction.

Injections of cultures of the Bacillus coli communis into animals produce variable and uncertain results. Subcutaneous injection may lead to pus-formation; in rabbits and guinea-pigs injections may produce death apparently from poisons introduced. With the blood of immunized animals a serum-reaction, similar to that described for typhoid fever, may be demonstrated.

Concerning the occurrence of the Bacillus coli communis in the intestine of man see page 164.*

At autopsies on human subjects the great viscera are often found to have been infected by the colon bacillus, usually when some lesion of the intestine existed simultaneously, but in most cases without having produced much apparent damage to the organs invaded. The Bacillus coli communis frequently occurs in mixed infections, as in wounds, inflammations and abscesses. It is often found in the peritoneum in peritonitis, in the pus in appendicitis, and in the urine in cystitis; it frequently occurs in the interior of gall-stones with whose formation it may be connected,† as first pointed out by Welch.

There is a large number of more or less closely related organisms which go by the name of the "*colon group.*" The limits of the colon group are extremely ill-defined.‡

Detection of Bacillus Coli Communis in Water.—To each of a number of fermentation-tubes containing 1 per cent. dextrose-bouillon add some of the suspected water (0.1 to 1 c.c. or more). Place in the incubator. Each day mark the amount of gas that has formed in the closed arm. After two days B. coli communis should render the bouillon strongly acid and produce about 50 per cent. of gas (30 to 70 per cent. according to different writers). The gas is approximately H two parts, and CO_2 one part (see page 132). From tubes showing these characters plates may be made and the usual tests for the colon bacillus applied.§ (See Part IV.) Stokes recommends adding the water to fermentation tubes containing 1 per cent. lactose-bouillon and neutral red (10 c.c. of a 5 per cent. solution of neutral red to a liter of bouillon); if the colon bacillus is present, 30 per cent. to 50 per cent. of gas is formed (con-

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*See also Moore and Wright. Bacillus coli communis in the Domesticated Animals. *American Medicine.* March 29, 1902.

†Lartigau. *Journal American Medical Association.* April 12, 1902.

‡Rivas. *Journ. Med. Res.* XV. 1906. pp. 397–509.

§Theobald Smith. *American Journal Medical Sciences.* Vol. CX. 1895.

sisting of one part of carbon dioxide and two parts of hydrogen), and the neutral red in the closed arm changes to a yellow color.*

Jackson† advises the use of bile as an inhibiting agent for the bacteria other than the intestinal bacteria found in water. The restraining action of the bile is due to the colic acid radical of the bile. Jackson recommends that undiluted ox-bile be employed. This is sterilized as soon as it is drawn, and may then be kept in stock. When used it should be decanted or filtered, and one per cent. of lactose previously dissolved in a small amount of water should be added. It should then be distributed into fermentation tubes 140 mm. long by 15 mm. in diameter having an elongated bulb 38 mm. in the shortest diameter. Instead of fresh bile, 110 grams of dried bile and 10 grams of lactose dissolved in 1 liter of distilled water may be employed.

Swin‡ recommends this medium particularly for sewage and for badly contaminated water.

The following scheme for the detection of *B. coli* in water has been recommended by Longley and Warren.§

Preliminary incubation in dextrose-broth fermentation tubes at 40° C. for 24 hours. Those showing gas are to be plated on litmus-lactose-agar. No note need be made of the reaction nor of the amount of gas. The above plates are to be incubated at 40° C. for 18 or 24 hours, and inoculations made on agar slants from any red colonies that may be found. The agar slants shall be incubated at 40° C. for 24 hours, and further tests made in dextrose-broth fermentation tubes. Tubes developing no gas are noted as negative. Milk tubes should also be inoculated from the agar slants and incubated at 40° C. for two days, and examined for coagulation both days. Failure to coagulate or digestion of casein after coagulation are to be regarded as negative. Cultivation for two days at 40° C. in nitrate-broth and testing for the presence of nitrites at end of this time. The presence of nitrites is regarded as positive. Cultures from the slant agar in peptone broth are incubated for 3 days at 40° C. and then tested for the presence of indol. The presence of indol is regarded as positive. The above statement is a somewhat condensed statement of Longley and Warren's scheme.

Paracolon or paratyphoid bacilli are the names applied to certain members of the colon group which have recently been shown to be pathogenic to man. They may produce clinical symptoms resembling typhoid fever of a mild and atypical form, but Wells and Scott‖ found that there is little to distinguish the pathological lesions produced by the paracolon bacillus from other septicemias. The intestinal lesions are quite variable, but there are no changes in the Peyer's plaques or solitary follicles. The intestinal glands are little or not at all affected. The ulcers of the intestines are not always present and when they are they differ from those found in typhoid fever. Probably they may occur with typhoid fever in mixed and secondary infections. Characteristic lesions have not yet been observed. The most constant change is splenic enlargement. The affection is rarely fatal. The bacilli have been found in the blood, spleen, liver, gall-bladder and urine. Like typhoid and colon bacilli they are motile, have flagella, are not stained by Gram's method and do not liquefy gelatin. They ferment dextrose and maltose, producing acid and gas. They do not ferment lactose. Milk at first becomes acid, later it becomes alkaline, and is not coagulated. On potato a slight visible growth occurs. Media contain-

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**Journal of Infectious Diseases.* I. 341.

†*Journal Infect. Diseases.* Supplement No. 3. May, 1907. pp. 30–32.

‡*Ibid.* pp. 32–38.

§*Journ. Infect. Dis.* Vol. IV. 1907, pp. 397–415.

‖*Journ. Infect. Dis.* Vol. I. 1904. pp. 72–90.

ing neutral red become yellow, as with B. coli communis, but more slowly, and the red color sometimes returns. In respect to the fermentation of saccharose and the formation of indol reports differ; both are usually negative. The blood of the patient agglutinates the bacilli. But, as among the closely related members of this group mutual reactions are sometimes seen, this test is not to be considered invariable.* Several bacilli allied to the above are known. The Bacill usenteritidis of Gaertner is a related form which has been found in cases of meat-poisoning.

Bacillus Lactis Aerogenes (Bacillus aerogenes).—A bacillus having a form similar to that of the colon bacillus, described as being larger and plumper. In the main its properties are similar to those of the colon bacillus. Its colonies are more circumscribed and elevated than those of the colon bacillus. It is non-motile. It coagulates milk more rapidly than the colon bacillus. It produces gas upon potato more rapidly than the colon bacillus, and more abundantly. It was first described by Escherich, who discovered the colon bacillus, assigning the Bacillus lactis aerogenes rather to the upper part of the small intestine, and the colon bacillus to the lower portion. According to Kruse, the Bacillus lactis aerogenes and its relatives differ from the Bacillus coli communis chiefly in lacking motility. Like the colon bacillus it has been found many times in the urine in cystitis. See also B. acidi lactici, page 273.

Bacillus Dysenteriæ (Shiga).—A bacillus with rounded ends, of the size and shape of typhoid and colon bacilli, seldom forming threads. Most observers have found it non-motile. Vedder and Duval have demonstrated flagella. The bacillus does not form spores. It may be stained with the ordinary aniline dyes; it does not stain by Gram's method. It is a facultative anaerobe. It grows at ordinary temperatures, but better in the incubator. It grows on the usual culture-media, but more slowly than B. coli communis. The growths are

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*Crushing. *Bulletin Johns Hopkins Hospital.* July–August, 1900. Strong. *Ibid.* May, 1902. Johnstone, Hewlett and Longcope. *American Journal Medical Sciences.* August, 1902. Libman and Buxton. *Journal Medical Research.* Vol. VIII. 1902.

whitish. Colonies on gelatin plates resemble those of the typhoid bacillus. Bouillon is diffusely clouded; a precipitate may form, but no pellicle. Indol is not produced. Milk becomes acid and is not coagulated. On potato a thin pale layer forms which may become light brown. No gas is formed in media containing glucose or lactose.

Neutral-red agar is not changed. From the feces the bacillus is best cultivated on agar plates, in the incubator. Colonies of B. coli communis are often more numerous than those of the dysentery bacillus. The colonies which develop in twenty-four hours are likely to be colonies of B. coli communis. The position of these may be marked on the glass with a pencil. Those which appear later are to be planted in dextrose-agar. If gas develops, they are not the bacilli of dysentery; otherwise they are to be studied and identified by the cultural and other tests mentioned above, and by the agglutination reaction.

They have been found in the intestine and the discharges of acute and epidemic dysentery in various climates and countries, including the United States. Thus far their dissemination in the blood and distant organs has not been demonstrated. The lesion of this form of dysentery consists of a severe acute inflammation of the colon, frequently with necrosis of the surface and the formation of pseudomenbrane. Ulceration may occur, but is usually superficial. Duval and Bassett found the bacillus of dysentery in the stools of infants having summer diarrhea; but Collins* failed to find B. dysenteriæ or paradysenteriæ in infants suffering from acute and subacute diarrhea.

The introduction of pure cultures into animals by way of the alimentary canal has sometimes been followed by a certain amount of diarrhea, but it does not appear that dysentery, as it occurs in man, has been reproduced. Most labor-

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**Journ. Infect. Dis.* Vol. II. 1905. pp. 620–626.

atory animals are, however, very sensitive to the injection into the tissues or veins of cultures, living or dead. They show the lesions produced by various toxins, but nothing of a characteristic nature.

The bacillus is agglutinated by the patient's blood, but often only late in the disease and apparently not in all cases. This test seems to have only a limited value in clinical diagnosis. Many prefer to secure the reaction in a test-tube. The dilutions used vary greatly (from 1 in 20 to 1 in 100). Immunized animals develop the agglutinins in the blood. Results of experiment made for the production of a curative serum are encouraging.

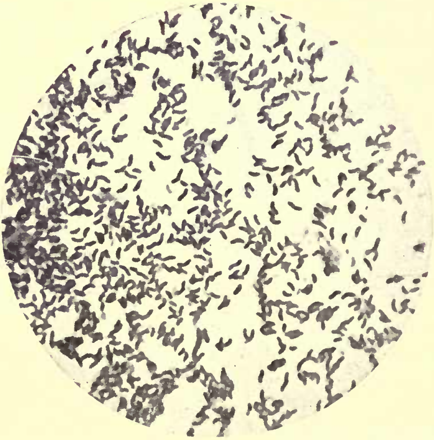

FIG. 100.—Spirillum of cholera.* (× 1000.)

Torrey† comes to the conclusion that the group of dysentery organisms is a large and varied one which may be divided into two groups: the Shiga-Kruse group on the one hand and the manite-fermenters on the other. Among the manite-fermenters, of which bacillus "Y" of Hiss and Russell and the Flexner-Manila bacillus are the type, there is great variation in cultural properties, but have this in common that they all produce the same change in litmus-milk. Flexner suggests that these organisms may be occasionally or constantly present in the normal intestines. Duval found them in the intestinal contents of children suffering from mild summer diarrhea. On the other hand the Shiga-Kruse type from some twenty different sources all reacted alike in the various culture-media and all agglutinated alike with various sera. According to W. H. Park, some of the manite-fermenters form indol which the bacillus of Shiga does not; they also differ from it in their agglutination reactions.*

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*The specimen from which this photograph was made was stained for flagella. A field was selected where the organisms failed to show flagella. But the method of staining probably accounts for the bacteria appearing somewhat thicker than they usually do in preparations.

†*Journ. Exper. Med.* Vol. VII. Feb.–Nov., 1905. pp. 365–384.

While it seems probable that the Shiga bacillus, and its congeners cause certain forms of dysentery, Jürgeus states that there is evidence to show that there are epidemics of dysentery which are caused by organisms which can scarcely be identified with them.†

It must also be borne in mind that epidemics of dysentery occur mainly, though not exclusively, in tropical countries which are caused by amebæ (see page 430).

Bacillus Pseudodysentericus.—Muller‡ gave the name B. pseudodysentericus to a type of organisms bearing all the cultural characteristics and pathogenic properties for experiment animals which are shown by B. dysenteriæ, and differing from this organism or group of organisms in one respect only; that is in the failure to agglutinate with blood-serum of persons suffering with dysentery. While stating that this classification of the organism is purely arbitrary and

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*Shiga. *Centralblatt für Bakteriologie.* Bd. XXIV. 1898. Flexner. *Philadelphia Medical Journal.* September 1, 1900. Vedder and Duval. *Journal Experimental Medicine.* Vol. VI. Gay. *University of Pennsylvania Medical Bulletin.* November, 1902. Duval and Bassett. *American Medicine.* Vol. IV. p. 417. 1902. Park and Carey. *Journal Medical Research* Vol. IX. 1903. Strong and Musgrove. *Journal American Medical Association.* Vol. XXXV., p. 498. 1900.

†Jürgens. Deutsche Med. Wochenschr. XXIX., No. 47. p. 1713.

‡Ford. The classification and distribution of the intestinal bacteria in man. Studies from Rockefeller Institute. Vol. II. 1904. pp. 1–95.

artificial in one place in the article referred to, Ford states in another place, that the failure to agglutinate with dysenteric serum positively differentiates the B. pseudodysentericus from B. dysenteræ.

Below are some of the characteristics noted by Ford.*

Bacilli measuring 0.5 by 1–2 microns in dimensions; in pairs and short chains. Slow motility in young broth and in agar cultures, motility more marked in older cultures. Spores absent. On agar slant growth confined to the line of inoculation. Deep colonies in agar round, regular and opaque. In general the colonies resemble those of B. typhosus. Growth in broth abundant with heavy sediment, but no pellicle. On gelatin no liquefaction. Fermentation in dextrose broth grow in both arms of the tube, reaction in closed arm, acid; no gas. Indol produced rarely in small quantities. It is found in the lower portions of the intestines, but also occurs in the stomach and duodenum as well, in persons not affected with dysentery. Does not agglutinate with the serum from persons suffering with dysentery.

Torrey† summarizes the points of difference between the true dysentery bacillus and the pseudo-forms as follows: The latter spread over the surface of the culture plate more than the B. dysentericus, they produce finally acidity in litmus milk, do not absorb agglutinins of B. dysentericus. The realtionship between the B. dysentericus and the pseudo-forms is about the same as that existing between the B. diphtheriæ and the pseudo-diphtheria bacilli.

Spirillum Choleræ Asiaticæ (Comma Bacillus of Cholera).—A rod-shaped organism, with rounded ends. It is usually curved, hence the name comma bacillus often given to it; but the curve is often very slight. The curved forms, placed end to end, may produce an S-shaped body. The length is from 0.8 to 2 μ and the breadth from 0.3 to 0.4 μ. In cultures some individuals may develop into genuine spirilla. In the whitish particles found in the stools of cases of cholera the organisms may be present in very large numbers. In these particles they may exhibit a very curious arrangement, lying parallel with one another, and, as remarked by Koch, they resemble a school of fish moving up stream. Involution forms, irregular in outline and staining poorly, are often seen in old cultures. The organism is motile, having a flagellum at one end. It does not form spores. It stains with the ordinary aniline dyes, but not by Gram's method. It is aerobic. It grows at the room-temperature, but better in the incubator. On the ordinary media the growths are whitish. It grows best on neutral or

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***Loc, cit.*

†*Journ. Exper. Med.* Vol. VII. 1905. pp. 365–400.

alkaline media, and is very sensitive to a small amount of acid. It liquefies gelatin. The colonies on gelatin plates have a very characteristic appearance. They are nearly round at first, and granular as seen under the low power of the microscope; but at the end of about twenty-four hours the outline is slightly irregular, and the surface looks as though it were covered with finely broken glass. The outline later becomes still more irregular or scalloped. As liquefaction of the gelatin takes place a funnel-shaped depression is formed, into which the colony sinks. Gelatin plates should be kept at a temperature of from 20° to 22° C. In stab-cultures in gelatin a white growth forms around the stab, and at the end of about thirty-six to forty-eight hours a funnel-shaped depression occurs on the surface, owing to the liquefaction of the gelatin. This depression increases in size, and the surface of the liquefied gelatin seems to be surrounded by an air-bubble, which appears to have taken the place of the part of the fluid gelatin which has evaporated. In the deeper portion o the stab liquefaction is less noticeable. The growths on agar are not characteristic. In bouillon a pellicle forms on the surface. On potato in the incubator the growth is whitish or brownish, not conspicuously elevated. After growing it in Dunham's

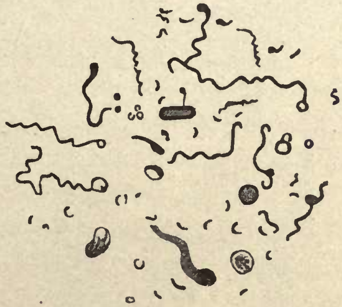

FIG. 101.—Involution forms of the spirillum of cholera.—(*Van Ermengem.*)

peptone solution in the incubator the addition of sulphuric acid develops a red color, owing to the presence of indol and nitrites—the so-called "*cholera red*" reaction. Considerable doubt has recently been cast upon the formation of nitrites by the cholera spirillum.* The cholera-red reaction is not confined to this organism, and is said to differ from the nitroso-indol reaction.

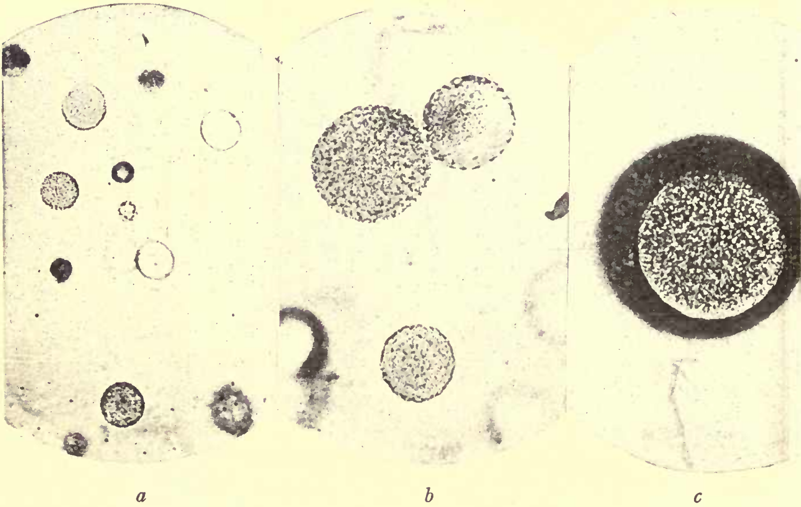

FIG. 102.—Spirillum of cholera, colonies on gelatin plates. (× 100 to 150.) (*Fränkel and Pfeiffer.*)
a. Twenty-four hours old. *b.* Thirty hours old. *c.* Forty-eight hours old.

The spirillum of cholera is said to be very sensitive to drying, and, provided the drying be complete, is usually killed within twenty-four hours. It is killed† immediately by the temperature of boiling water, in 5 minutes with certainty at 80° C., in one hour at 56° C. It may retain its vitality in water for a long time; observations vary widely in respect to deter-

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*Wherry. *Journal of Infectious Diseases.* Vol. II. No. 3. June 24, 1905.
†Kolle in Kolle and Wassermann. Vol. III. 1903. p. 23.

mining how long. In the ordinary food-substances it may survive long enough to allow them to act as carriers of the infection if eaten raw. It is an important fact that the cholera spirillum is not a strict parasite, but under favorable conditions it may maintain its vitality for some time outside of the human body.

The animals ordinarily used for laboratory experiments are, in their normal condition, not susceptible to infection with the spirillum of cholera through the alimentary canal, and no animal is known which suffers from spontaneous cholera excepting man, though a disease resembling cholera can be reproduced in animals when certain conditions are complied with. The acid of the gastric juice destroys the organism, and this makes it impossible to infect animals by way of the alimentary tract unless this acidity is overcome with an alkali before the introduction of the culture.

The following plan was adopted by Koch: The gastric juice was neutralized with a solution of sodium carbonate; the movements of the intestines were quieted by the injection of 1 c.c. of tincture of opium for each 200 grams of the body-weight; and a portion of pure culture of the cholera spirillum was introduced into the stomach. When guinea-pigs are treated in this manner, in most cases a condition closely simulating cholera is produced. The animal dies with symptoms of collapse. The small intestine is more or less filled with a watery, flocculent fluid containing a large number of the spirilla of cholera. The mucous membrane of the intestine is swollen and reddened.

FIG. 103.—Spirillum of cholera, stab-culture in gelatin, two days old. (*Fränkel and Pfeiffer.*)

When guinea-pigs receive an intraperitoneal injection from a pure culture, death usually results, apparently from the toxic substances contained in the culture. Pfeiffer was the first to show that an animal may be made immune from cholera by repeated small doses of cultures which have been heated in order to kill the organism. He also showed, in the same connection, that when living comma bacilli are introduced in the peritoneum of an immune animal they first clump together and are then rapidly destroyed and disintegrated (see page 228); furthermore, that a drop of the peritoneal fluid added to a hanging-drop culture of the cholera spirillum produces the same effect. This is now called Pfeiffer's phenomenon, and is the underlying principle of all agglutination reactions, such as the Gruber-Widal typhoid test.

It seems probable, from the results so far obtained, that it is practicable to use injections of dead cultures upon human beings with safety, and in this way so protect healthy persons from cholera during an epidemic.*

Haffkine† elaborated a special method which goes by his name. He found that by passage through guinea-pigs the virulence of the culture could be exalted to a constant maximum point which he calls his "virus fixe." This is attained by a passage through 20 or 30 guinea pigs. The guinea-pigs are all injected into the peritoneal cavity, starting by inoculating a guinea-pig with a dose larger than is necessary to kill the animal, and inoculating the subsequent animals of the series with the peritoneal exudate of the dead animals. The exudate must be exposed to the air for 15 hours before it is injected into the next guinea-pig, otherwise it is found to be not so virulent, this exposure contrary to usual experience enhancing the virulence. Small guinea-pigs give less abundant but more concentrated exudate. This so-called "virus fixe" is cultivated in beef-broth at 39° C. in a current of air, and kept in this manner until cultures made daily on agar fail to show any growth. The last culture which grows injected into a guinea-pig subcutaneously does not produce any necrosis, and it also prevents the necrosis of the skin produced by "virus fixe" when so injected. Haffkine inoculated himself with the attenuated culture described, and 6 days later with the "virus fixe;" he then proceeded to inject a large number of persons, and finally to try its inoculations upon more than 100,000 persons in localities in India, with apparently somewhat favorable results. The inoculation does not modify the course of the disease in those cases in which an inoculated person contracts the disease. The percentage of death is as great among such persons as among uninoculated persons who contract the disease.

Although a positive demonstration that the spirillum of Koch is the cause of cholera is lacking, as far as the exact

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*Strong. *American Medicine.* August 15, 1903.

†*Bull. de l'Inst. Past.* Vol. IV., Nos. 17 and 18. 1906. pp. 697–705, and 737–747.

reproduction of the disease in animals is concerned, the necessary proof has been supplied by the accidental or intentional infection of laboratory investigators who were working with cholera, which has happened on several occasions.

Bacteriological investigations have shown that the spirilla of cholera are present in very large numbers in the watery contents of the intestine, especially early in the disease. They appear in the lumina of the glands, and they may be seen underneath the epithelial cells. They may occur in the matters vomited. They have been found in the feces of apparently well persons in times of cholera epidemic. These persons may spread the disease, and are to be regarded as bacillus carriers just as in the case of typhoid fever where healthy persons harbor the bacilli and evacuate them in the stools. They usually are not found widely spread through the organs of the body. It is probable that the symptoms of the disease result from poisonous substances produced by the spirilla or contained in them.

The portal of entry in cholera is probably always the alimentary tract, and the infectious agent is usually, though not always, transmitted through drinking-water, and numerous epidemics have been traced to this source. In some cases the origin of the contamination of the water with cholera dejecta has been demonstrated. The organism may, however, be introduced into the alimentary tract upon any and every article of food. It may be conveyed from place to place upon soiled clothing and bedding, and then be brought in contact with food. Flies also probably convey the organisms from cholera stools to articles of food. In order to combat the spread of the disease the excreta and bedding should be thoroughly sterilized; the hands of the attendants should be carefully disinfected and all food should be cooked. Although commoner in the summer-time, epidemics of cholera have been known to occur in the winter.

Bacteriological Diagnosis of Cholera.—When cases suspected of being cholera appear in a community, it becomes a matter of the utmost importance to determine the exact nature of the disease in order that it may not become epidemic. One of the first occasions when bacteriological methods were put into practice in the diagnosis of cholera was at the time of the appearance of that disease in the port of New York in 1887.

According to Koch, the diagnosis may be made in twenty-four hours or less. It is important to obtain the discharges from the intestines as early in the course of the disease as possible, and while they are perfectly fresh. It may be necessary, however, to examine the moist dejecta on the linen or clothing, when no other material is available.

In the first place, one of the small, partly solid particles which may be found in the discharges from the intestines should be smeared upon a cover-glass, fixed in the usual manner, stained with one of the aniline dyes, and examined with the microscope. If taken early in the disease, the comma bacilli may be present in large numbers, and they are likely to be arranged in parallel groups, as already described. If comma-shaped bacilli are thus found, a strong probability is created that the disease is Asiatic cholera. The motility of the organisms can be determined by examination in the hanging-drop. It is to be remembered that spirilla of various forms are common in the normal mouth, and may appear in the stools (see pages 161 and 274).

The method of procedure recommended by Koch, Kirchner and Kolle,* sent out by the German minister of medical affairs to the different directors of hygienic institutes in Germany for their guidance is in substance as follows:

Microscopic examination of the stools, preferably of the mucous flakes should be made by smearing the material over

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*Kolle and Wassermann. Bd. III. 1903. p. 42 *et seq.*

the cover-glass in the usual manner, and staining with carbol-fuchsin diluted 1 to 9. Also microscopic examination of hanging drops prepared in peptone solution. These preparations are made and examined immediately and after 30 minutes in the incubator at 37° C., also dried and stained. The peptone solution is described below.

Gelatin plates should also be made from the same material. These should be made in 2 series of 3 plates each. The first tube of melted gelatin is inoculated with a loopful of the stool, preferably from a mucous flake. The other tubes are inoculated in the customary manner, 3 loops from the first to the second, 3 loops from the second to the third. The plates should be placed at 22° C. for 18 hours and then examined with the low power of the microscope. Impression preparations and smears should also be examined, and pure culture made from colonies.

Agar plates should also be made in the manner described for gelatin plates, or the loop of material from the stool may be stirred up in 5 c.c. of bouillon, and three plates made from this suspension, using one loopful for each plate.

Forthermore, 6 tubes, each with 10 c.c. of the peptone solution described below, are inoculated each with 1 loopful of the feces, and examined with the microscope after 6 to 12 hours in the incubator at 37° C. without shaking up the contents of the tube. The tube showing organisms most resembling cholera is used for the purpose of making plates in agar and in gelatine as described above for plates directly from the stool.

In addition to the plates, 3 tubes of peptone are inoculated, each with 1 loopful. The peptone tubes should be warmed to 37° C. before they are inoculated.

Lastly, a flask containing 50 c.c. of the peptone solution is inoculated with 1 gram of the stool, and examined of 6 to 12 hours as above.

The peptone solution is made by taking 1 part of the enriching fluid described below for the examination of water for the cholera spirillum (p. 399), and diluting with 9 parts of water, distributing it in test-tubes, 10 c.c. in each, and sterilizing.

At the time that the first smear preparations and gelatin plates are prepared, tubes of peptone solution should be inoculated directly from the intestinal contents, and kept in the incubator (Schottelius). After development has occurred, the production of indol may be tested by the addition of sulphuric acid. These tubes are especially valuable when unfavorable material or *when material containing small numbers of the spirilla is used.* In the incubator the spirilla may be expected to multiply in the peptone solution rapidly, and to appear upon the surface of the liquid in large numbers, even forming a visible film in six hours. Smears may be made from the surface part of these tubes, stained, and examined with a microscope. From the same material gelatin plates should be prepared, and examined as soon as the colonies develop.

Ashburn and Craig* have sounded a warning against too great reliance upon bacteriological results in the early diagnosis of cholera. It is seldom that even approximately pure cultures of the cholera bacillus are obtained from stools, and from mixed cultures from the stools they obtained the "cholera-red" reaction in one case only. The reaction can usually be obtained in pure cultures by using media prepared with Grübler's peptone, but not with other peptones.

When cultures are obtained, their effects may be tested upon guinea-pigs, by injecting them into the peritoneum.

The production of Pfeiffer's phenomenon is at present regarded as the most important and final means of diagnosis between the cholera spirillum and related forms. This con-

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**Journ. Am. Med. Assn.* Vol. XLVIII. No. 8. Feb. 23, 1907. p. 692.

sists in testing the suspected organism with serum from an animal immunized with cultures of cholera bacilli, as already explained above.

The serum employed in this test must of course be prepared by injecting the animal furnishing the serum with pure cultures of a strain of undoubted origin. The potency of the serum itself must be determined by trial upon cultures of known identity. This is done* by preparing a series of dilutions of the serum with normal salt solution in test-tubes, each test-tube to contain 1 c.c. of the diluted serum. To each of the series of tubes one loopful from an eighteen-hour agar culture is added, and the tubes so prepared are placed in the incubator at 37° C. for one hour. The strength of the serum is denoted by the dilution which causes an agglutination which can be seen with the naked eye. Thus a serum of the strength 1–1000 is such that 1 c.c. of a dilution of 1 part of serum to 999 parts of normal salt solution causes a visible clumping of 1 loopful of the cholera spirillum taken from an 18 hour agar culture. If the strength of the serum is thus determined, and if a culture obtained from the stool of a person presenting suspicious symptoms of cholera, it is safe to make a positive diagnosis.

For examining suspected water for the spirillum of cholera Kolle† advises the following method: An enriching fluid is first prepared which consists of 1 liter of distilled water in which are dissolved by warming 100 grams Witte's peptone, 100 grams salt, 1 gram potassium nitrate, and 2 grams crystallized carbonate of sodium. The solution is filtered, and distributed into flasks, 100 c.c. in each flask, and sterilized.

At least 1 liter of the water to be examined is taken, and to it is added the contents—100 c.c.—of one of the flasks of enriching fluid. It is then distributed into flasks, 100 c.c. in each flask, and put in the incubator at 37° C. and examined in from 8 to 18 hours. The examination consists in the microscopic examination of the scum which forms more or less on the top of the fluid—the flask showing the most scum is selected—and in the application of the various tests for the identification of the organism given above. See also page 145.

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*Kolle and Wassermann. Bd. III. 1903. p. 39.

†Kolle and Wassermann's Handbuch. Vol. III. 1903. pp. 44–45.

Since Koch's discovery of the cholera spirillum in 1883–84 a considerable number of bacteria have been described which resemble the cholera spirillum more or less closely, and these have to be taken into account in making examinations of suspected material of any sort. This is particularly necessary in the investigation of water, in which such cholera-like spirilla seem to occur quite frequently.

Vibrio Metchnikovii.—A comma-shaped organism, which, though somewhat shorter and thicker than the cholera bacillus, is very similar to the latter in form, and, like this, may sometimes form genuine spirilla. It is motile and has a flagellum at one end. It does not form spores. It is aerobic. It stains with the aniline dyes, and is not stained by Gram's method. It grows at the room-temperature. It liquefies gelatin somewhat more rapidly than the spirillum of cholera. The colonies on gelatin plates are not all alike; some of them resemble those of Vibrio proteus, and others are extremely like those of the spirillum of cholera. It grows upon the usual media. Coagulated blood-serum is liquefied by it. The growth on agar is grayish to yellowish, and abundant. It forms a pellicle on bouillon. In milk an acid reaction is developed with coagulation. In peptone solution it produces indol and nitrates like the spirillum of cholera. It is said to give the nitrosoindol reaction more intensely than the spirillum of cholera.

It was discovered in chickens suffering from gastro-enteritis. It is pathogenic for chickens, pigeons and guinea-pigs; less so for mice and not at all for rabbits.* The comma-shaped

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*Günther. *Loc. cit.* p. 683.

organisms are found in the blood in guinea-pigs, pigeons and young chickens.

Vibrio Proteus (Finkler and Prior).—A comma-shaped organism somewhat larger than the spirillum of cholera, sometimes exhibiting genuine spiral forms, and also, at times, involution forms. It is motile and has a flagellum at one end.

The developments of the colonies in gelatin and the liquefaction of this medium are more rapid than with the cholera spirillum. At the end of twenty-four hours the colonies are all circular, larger than those of the spirillum of cholera, and uniformly granular when slightly magnified. On the other culture-media the growths are usually whitish. On potato it produces an abundant, moist, grayish-yellow deposit, and grows at the room-temperature. It liquefies coagulated blood-serum; milk becomes acid. In peptone solution is does not usually form indol, but occasionally it does so. It is less pathogenic to animals than the spirillum of cholera. It was

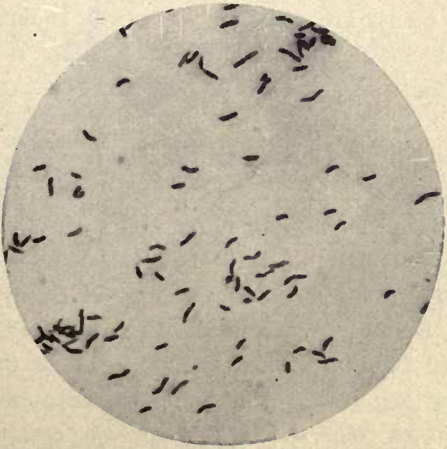

FIG. 104.—Vibrio proteus.*

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* The magnification is a little greater than in the other photomicrographs.

supposed by its discoverers to be the cause of cholera nostras, but it appears to have no relation to that disease.

Spirillum Millieri.—A comma-shaped organism resembling Vibrio proteus in many respects, and probably identical with it. In gelatin it grows more rapidly, and produces liquefaction more rapidly than the spirillum of cholera. On gelatin plates, at the end of twenty-four hours, the colonies are uniformly circular and granular, lying in little depressions resulting from the liquefaction of the gelatin. Its growths in the other media are not characteristic. It liquefies blood-serum. It does not produce indol as a rule. It is less toxic to animals than the spirillum of cholera. It was isolated by Miller from a carious tooth.

See also Spirillum sputigenum, Part III.

Spirillum Tyrogenum (Deneke).—A comma-shaped organism, not so large as the spirillum of cholera. It is motile, having a flagellum at one end. It does not form spores. In cultures, genuine spirilla may develop. Gelatin is liquefied more rapidly than by the spirillum of cholera, and the colonies develop more rapidly. The circumference of the colony is round, the surface may appear somewhat granular, and it has a greenish-brown color, seen under the low power. Milk containing litmus becomes acid, is subsequently decolorized, and is also coagulated. It liquefies coagulated blood-serum. It does not form indol in Dunham's peptone solution as a rule. No pellicle forms in cultures upon bouillon. It is less toxic to animals than the spirillum of cholera. It was isolated originally from old cheese.

Vibrio Berolinensis.—A comma-shaped organism resembling the spirillum of cholera in form and in the position of its flagellum. It does not stain by Gram's method. It grows at the room-temperature, but more rapidly in the incubator. The colonies upon gelatin, one or two days old, when magnified, are decidedly more finely granular and more transparent than those of the spirillum of cholera, and the margin is almost absolutely smooth and circular. As the colonies become older they assume a more irregular and lobulated appearance, but are still more finely granular than the colonies of the cholera spirillum. Gelatin is very slowly liquefied. Its growth on the other culture-media is not remarkable. It forms indol in peptone solution, and it increases in the upper layers of the fluid.

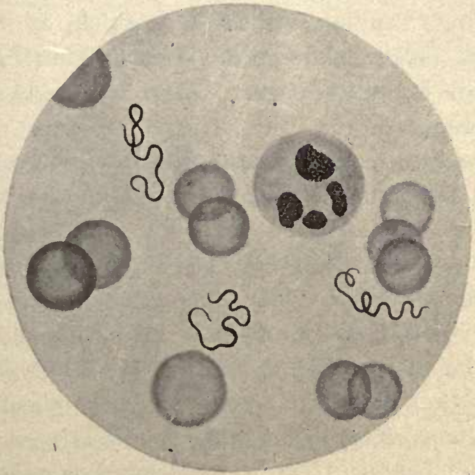

FIG. 105.—Spirillum of relapsing fever in the blood. Sketched from a stained specimen.

When guinea-pigs are inoculated in the peritoneal cavity, death occurs in one to two days. This organism was discovered in the water-supply of Berlin.

Other spirilla have been isolated from water by Günther (Vibrio aquatilis in Spree water); by Dunbar from the Elbe River; by Russell from the Gulf of Naples; by Heider from the water of the Danube Canal; and in America, by Abbott, from the water of the Schuylkill (Vibrio Schuylkiliensis); and many others have been described to which the limits of this work will not permit of further allusion.

Two vibrios, V. Massaua and V. Ghinda, were isolated in the places after which they are respectively called by Pasquale.* Both were held to be genuine cholera spirilla at first, but the failure to agglutinate with cholera-immune serum differentiates them from this organism. V. Massaua was isolated from the dejecta of a patient who was suffering from some other disease than cholera, though there had been an outbreak of cholera at the place before Pasquale's arrival. The other vibrio was isolated from drinking water. Gotschlich 21 cultures of vibrios from various material in Alexandria, in Egypt, all of which could be differentiated from cholera by means of immune serum.†

The Spirillum or Spirochæta Obermeieri (of Relapsing Fever).—A slim spirillum with numerous turns, 16 to 40 μ in length. The ends are pointed. It is actively motile. The spirillum is not stained by Gram's method, but may be colored by the ordinary aniline dyes. The organism has never been cultivated. It is found abundantly in the blood and in the spleen during the attack of fever. The spleen is enlarged. The disease has been produced in apes by inoculating them with blood taken from men having the disease.

Novy and Knapp‡ came to the conclusion as the result of their investigations in this direction that the S. obermeieri is to be classed with the bacteria and not with the protozoa. They also found that besides human beings, monkeys, white mice, rats; wild and tame are subject to infection with the organism; moreover, monkeys, mice and rats can be promptly cured by injections of hyperimmunized blood. S. obermeieri can be made to pass through a Berkefeld filter.

In a preliminary note Novy and Knapp§ report the cultivation of a spirillum which they call S. obermeieri with reserve;

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* Kolle and Wassermann. Bd. III. 1903. p. 71.

†*Ibid.* p. 72.

‡*Journ. Infec. Diseases.* Vol. III. 1906. pp. 291–393.

§*Journ. Am. Med. Assn.* No. 26. Vol. XLVII. Dec. 29, 1906.

for although it was obtained from a case of relapsing fever, they have grounds for the belief that the organism causing the disease in America differs from that found in the eastern disease. The method employed consists in the use of collodion sacs filled with rats' blood. These sacs are inoculated with a small amount of blood containing the spirillum, and are then placed in the peritoneal cavity of white rats.

Spirochæta Pallida.—First observed by Schaudinn and Hoffmann* in recent as well as more advanced syphilitic lesions, on the surface and deep in the tissues in chancres, indolent buboes and papules. These observations have been abundantly corroborated, but for diagnostic purposes they can scarcely yet be employed with certainty on account of the difficulty of recognizing the spirochæta in exposed lesions such as mucous patches.

Flexner† finds that silver impregnation often brings out many more spirals than the anilin dyes. He furthermore finds that unexplained factors affect the stain with anilin dyes, and that the best results are to be obtained with Stern's‡ method of staining with silver.

It is asserted that the spirillum is transferred by bed-bugs from one person to another.§

Grouven|| succeeded in producing a disease with lesions characteristic of syphilis in a rabbit by intraocular inoculation. S. pallida was demonstrated in some of the lesions in the rabbit.

Levaditi and McIntosh¶ claims to have cultivated an organism identical with S. pallida in all respects, except that it was

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*Schaudinn and Hoffmann. Vorläufiger Bericht über das Vorkommen von Spirochæten in syphililischen Krankheitsprodukten und bei Papillomen *Arbeiten aus dem Kaiserlischen Gesundheitsamt.* Bd. XXII., p. 527, 1905.

†*Journ. Exper. Medicine.* Vol. IX. 1907. pp. 464–472.

‡*Berl. klin. Wochenschr.* XLIV. 1907. p. 400.

§Karlinski. *Centralblatt für Bakteriologie.* Bd. XXXI. Original. 1901

||*Deutsche Med. Wochenschr.* Nov. 21, 1907.

¶*Ann. de l'Inst. Past.* Vol. XXI., No. 10. Oct. 25, 1907. p. 784.

non-pathogenic, by the use of collodion sacs placed in the peritoneal cavity of apes. Metchnikoff and Roux found the same organism in the monkeys which they had successfully inoculated with syphilis.

The evidence is accumulating rapidly in favor of this organism as the cause of syphilis.* It is 4 to 14 μ long, ¼ μ thick and has 6 to 14 turns. It is actively motile. Stained with great difficulty. The following stain was recommended originally, and more recently a variety of stains have also been employed by different observers:

(1) Three parts Giemsa's eosin solution (2.5 c.c. 1 per cent. eosin solution in 500 c.c. water).
(2) Three parts asur I solution (1 gram asur in 1000 c.c. water).
(3) Three parts asur II solution (0.8 gram in 1000 water).
Mix and stain dried cover-glass preparations from 16 to 24 hours; wash, dry and mount in balsam.

Spirochæta Refringens.—Found less frequently than S. pallida in the same locations as the latter. Is larger and stains more easily than S. pallida.

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*Spirobacteria in the Lesions of Syphilis. *Journal of the American Medical Association.* Vol. XLIV., No. 22. p. 1790. 1905.
Schultz. *Journ. Med. Res.* XV. 1906. pp. 363–381.

PATHOGENIC PROTOZOA.

Protozoa are unicellular animal organisms. They are attracting ever increasing attention since they have been found to play a very important part in the causation of diseases, particularly of diseases peculiar to tropical countries, and as they are studied by methods that have much in common with those used for the bacteria they may be considered here briefly. Protozoa are numerous in pond and ditch water, and these species seem to be harmless. However, many diseases of the lower animals are caused by protozoa, such as surra, Texas fever and coccidium disease of rabbits. Birds,* reptiles and frogs† may show organisms in the blood resembling the parasites of malaria. Until recently it has been doubtful whether any pathogenic protozoön has ever been propagated in pure culture outside of the body of the host. This has been accomplished by Novy and MacNeal for a parasite (Trypanosoma) from the blood of the rat‡ and from many species of birds§ on rabbit-blood-agar.

Novy|| gives the following classification of the pathogenic protozoa which are of most interest: Trypanosomata, met free in the blood plasma; hemocytozoa, found in the blood-cells, represented by the malarial parasite in man and by related organisms in the lower animals, also by the piroplasmata found in Texas cattle fever and allied affections; amebæ, found in the intestines in dysentery and elsewhere. Other forms of pathogenic protozoa have been recognized,

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*Opie and MacCallum. *Journal Experimental Medicine.* Vol. III.

†Langmann. *New York Medical Journal.* January 7, 1899. Williams and Lewis.

‡Novy and MacNeal. Contributions to Medical Research. Dedicated to Victor C. Vaughan. 1903.

§Novy and MacNeal. On the Trypanosomes of Birds. *Journal of Infectious Diseases.* Vol. II., No. 2. p. 257. March, 1905.

||Reprint from *Journ. Am. Med. Assn.* Jan. 5 and 12, 1907. Vol. XLVIII.

but these are as yet of subsidiary importance compared with those just named.

Trypanosomes.*—The trypanosomes are roughly spindle-shaped and approximately crescentic. Each cell has a sharp posterior extremity; the anterior extremity narrows into a single long flagellum which is in active motion during life. An undulating membrane surrounds the organism from near the posterior extremity to the base of the flagellum. This membrane can be seen with the microscope extending like a fin on either side. The undulating membrane probably constitutes the organ of locomotion; not the flagellum as in the bacteria; since in one species the flagellum is rudimentary or lacking and still the organism is endowed with the power of locomotion, and, moreover, in cultures in which the undulating membrane is poorly developed, but in which the flagellum is very long it may show scarcely any motion. The cells consist of nearly colorless, almost homogeneous protoplasm. When stained by the Romanowsky method various structures are brought out. The nucleus which is near the anterior end stains blue. The flagellum stains deep red. A body known as the micro-nucleus or centrosome or blepharoplast which is a prominent object near the posterior end, and which is connected with the flagellum by a distinct line passing in the undulating membrane along the side of the organism, in fact it is continued into the flagellum, is also stained deep red. The periblast may also take a red stain. The plasma stains blue.

The adult cell is 25 mikrons long by 1.5 wide, about 3.5 times as long and about one-fifth as wide as a red blood-corpuscle. Multiplication takes place by obliquely longitudinal division only. Transverse division has never been

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*Following descriptions of trypanosomes is adapted from Novy. *Loc. cit.* For very full account of trypanosomes previous to 1903, see Musgrave and Clegg. Bureau of Gov. Labs., Philippine Islands, No. 5. 1903. 248 pages.

observed. Conjugation has also not been observed at least satisfactorily. The blepharoplast is the first to begin dividing, the flagellum remaining attached to one of the resulting halves, while a new flagellum develops on the other half. The division of the nucleus follows next in order as a rule, though this may precede the division of the blepharoplast. The active division of the nuclei and plepharoplast give rise to rosette formation owing to delayed division of the cell protoplasm.

Cultivation of Trypanosomes.—Many trypanosomes are much alike morphologically so that they are hardly distinguishable with the microscope alone, but in cultures the different species present points of difference. The culture medium adapted to their growth consists of equal parts of defibrinated rabbit's blood serum and ordinary nutrient agar. The method of preparation of the medium consists in melting up tubes of agar and after cooling them down to 50° C. adding the blood-serum. The tubes are allowed to set in an inclined position, then stood upright to allowed the water which separates out to accumulate at the bottom of the incline. Tubes should be fresh, since it is necessary to have abundant moisture in the tube. After inoculation, the tubes are kept at 25° C. or in the room. First growth is observed in about three or four days to one week. Initial cultures are not so vigorous as subsequent cultures. Usually there is no growth visible with the naked eye, but sometimes the growth is evdent in this way. Pure growths may be obtained by making streaks on blood-agar plates. The forms met with in cultures differ from those seen in the blood of an infected animal. They are usually smaller in the cultures than in the blood, although sometimes they are much longer than these. The blepharoplast is usually, though not always, lateral or anterior to the nucleus in the cultivated forms. Some species, as Tr. avium, show two distinct forms in cultures giving rise to the suggestion of sexual difference. Trypanosomes found in the stomachs of mosquitoes, tsetse flies, house flies, ete., show forms like those met with in cultures.

From accumulating data it would appear likely that all species of animals may show trypanosomes in the blood. Novy and McNeal were the first to cultivate them in pure cultures. Herbert U. Williams and Lewis were the first to cultivate the frog trypanosome, Tr. rotatorium, and this furnishes an excellent example for class demonstration.

Tr. evansi is found in the blood of animals in India and the East, generally suffering from a disease called *surra*. Horses, mules, camels, dogs and cattle are particularly subject to the disease which is characterized by remittent fever accompanied by anemia and wasting, edema of the legs, belly and other parts, with a discharge from eyes and nose. The organism is transferred from sick to healthy animals through the agency of flies. The disease is seldom fatal.

Tr. brucei, almost indistinguishable from *Tr. evansi*, causes the tsetse fly sickness, *nagana*, of Africa, a most fatal disease of horses, donkeys, dogs, and cattle. Nearly all mammals are susceptible to spontaneous or to artificial infection. Man appears to be immune. Tr. *brucei* was the first pathogenic trypanosome which was cultivated artificially. Differs markedly in cultures from Tr. lewisi and Tr. evansi.

Tr. equiperdum causes the disease of horses, known as *dourine*. The disease is not confined to tropical countries, but is found in many parts of Europe as well. It is said also to be met with in America. It is not spread as other diseases which are caused by trypanosomes through the agency of flies; but only by sexual contact, and hence is called "mal du coit," it is also called horse syphilis owing to suggestive skin lesions. The diseases may be communicated to asses, dogs and rabbits. Rats, mice and guinea-pigs are refractory.

Tr. equinum causes the disease known as "mal de caderas," occurring almost exclusively in horses. It is characterized by remittent fever, rapid loss in weight, with eventually paralysis of the hind quarters. The organism has no blepharoplast which distinguishes it from all other trypanosomes. No definite success has attended efforts at cultivation of *Tr. equinum.*

Tr. dimorphon causes a disease of horses in Senegambia. No other domestic animal than the horse suffers spontaneously, but most mammals can be artificially infected.

Tr. theileri causes a disease of cattle in South Africa.

Tr. gambiensis is the cause of the sleeping sickness of West Africa. The disease is spread through the agency of the tsetse fly, though a different species of this fly from that which spreads the nagana of South Africa. The disease is characterized by two stages: A mild stage marked by mild symptoms consisting of some fever, slight edema and erythema; the second stage is the sleeping stage always terminating fatally. Most animals may be infected artificially.

Leishman-Donovan bodies are called with a question mark trypanosomes by Novy who states that they resemble rounded forms of trypanosomes, showing a nucleus and a micro-nucleus. They have no undulating membrane. They are called piroplasma by Laveran. Rogers regards them as belonging to the herpetomonas group and not to the trypanosomes. They are the cause of Kala-Azar, a cachexial fever of India.

Walker* has published a full account of his work on the cultivation of parasitic amebæ.

While giving due credit to the large amount of valuable work done by others in the study of amebæ, Walker has perfected the methods of study to such an extent as to make this branch of investigation now within the range of exact observation. Walker's original article is recommended to those who wish to persue the subject fully; but the following description taken from his paper may serve as a guide, and may perhaps enable the student to begin investigations into this subject which is increasing in importance daily.

Cultivation of Amebæ.—The medium best suited for the growth of amebæ is that introduced by Musgrave and Clegg† which consists of:

Agar	20.00
Sodium chlorid	0.3–0.5
Extract of beef	0.3–0.5
Distilled water	1,000

This medium is prepared as ordinary culture-media for the cultivation of bacteria, except that the reaction should be 1 per cent. alkaline instead of the 1.5 usually employed for bacteria. Ameba cultivated upon this medium will afterwards grow less readily than at first upon liquid media, but they grow more readily upon the solid medium than in liquid media when transplanted from the latter to the former. *Ameba fecalis* is the only one which grows on an acid medium. All ameba require the presence of bacteria in the culture-medium and it seems that the bacteria are either eaten by the ameba or that they transform the medium in some way so as to make it assimilable by the amebæ. Moisture is even more essential for the growth of amebæ than for that of the bacteria.

**Journ. Med. Research.* Vol. XVII., No. 4. (New Series, Vol. XII. Feb., 1908. pp. 379–457.)

†Dept. of the Interior. Bureau of Gov., Labs., Biolog. Lab., No. 18. Oct., 1904. Manila Bu. Pub. Printing.

The water which squeezes out of the agar when the latter solidifies is sufficient if it is not allowed to evaporate. A glass jar provided with a cover and containing a vessel holding water may be employed for slant cultures. For Petri dish cultures a battery jar inverted in a shallow dish of water will serve to keep the cultures moist. Oxygen is essential to the growth of the amebæ. They grow best at about 20° C. to 25° C. but they will also grow at 37.5° C.

ISOLATION OF PURE CULTURES OF AMEBÆ.

The amebæ grow only upon the surface of the solid medium, and they do not remain fixed as do the bacteria by the setting of the agar, but creep about from place to place. It is therefore necessary to resort to special methods to secure cultures consisting of one kind of ameba only. If an agar Petri plate previously poured and allowed to harden is stroked with a platinum needle in several parallel lines with material containing ameba the lines made last will show scattered individual ameba cells. The needle is dipped only once into the material to be studied. The plate is examined with the low power of the microscope, and a single ameba separated from all the rest is located. A high power lens is then turned into position and run down until it comes in contact with the ameba selected. In this way the ameba is picked up on the end of the lens and may be transplanted to fresh media to grow out and form a culture consisting of the progeny of this one cell. Another method of obtaining a pure culture of amebæ is to locate a cell as just described with the low power of the microscope but with the Petri dish inverted. The location is marked around on the bottom of the inverted dish with a wax pencil or with ink. The dish is now turned right side up, and the place marked out as the location of the cell is inclosed by drawing a ring of vaseline around it. This prevents the ameba from wandering out, and also prevents other species from wandering in. After isolation by method just described the cell is allowed to multiply where it is, and after it has done so, as shown by observation with the microscope, further culture may be made on fresh tubes or plates. These cultures are pure only in the sense that they contain only one kind of ameba, but they require the presence of bacteria for growth as has been elsewhere stated.

Study of the Amebæ on Cover-glasses.—Hill's hanging block method is not well suited to the purpose of studying the amebæ for the reason that these grow only upon the surface but agar spred in a thin film on a cover-glass answers the purpose well. The method of procedure is to spread the melted agar on the cover-glass, and protect it against contamination from the air by placing it under a flamed watch glass. The spreading is affected with a large platinum loop. The agar is inoculated with the ameba, and the cover-glass onto which it is attached is placed on a hollow slide in the manner used in making the usual hanging drop cultures of bacteria. After the vaseline is streaked around the hollow in the slide, the latter may be pressed down over the cover-glass containing the inoculated culture medium, or the cover-glass may be turned over on to the slide in the usual way.

Reproduction in the Genus Ameba.—Studied in the "hanging plate" method just described, ameba are seen to pass through several stages. The ameboid stage may give rise to other ameboid forms by division, or to a resting stage through encystment, or finally to the production of young amebæ through spore formation.

In the process of development first mentioned the cell comes to rest, becomes rounded and then oval, and finally becomes constricted into a mother and a daughter cell by transverse division. Sometimes the two cells remain attached by a thin thread of protoplasm which stretches to several times the diameter of the cells. The cells are sometimes pulled together by the retraction of this thread which thickens as it shortens. The cells may pull apart and be drawn together again by this thread of protoplasm several times in the course of an hour. Division of the nucleus is in some cases typically amniotic. By dropping cover-glasses in fixing fluid at various stages of the process certain stages can be studied. The encystment mentioned above takes place sooner or later in all culture after longer or sorter period of multiplication and independently of the amount of moisture, but drying out hastens the process. Encysted amebæ transplanted to fresh culture-medium usually begins to show signs of awakening activity in twenty-four hours. These consist of agitation of the granules of the protoplasm, and finally pulsation of the vacuole. If too much of the old bacterial growth is transplanted on to the fresh culture medium, the cysts may fail to germinate. No multiplication takes place within the cyst. The ameba leaves the cyst behind as an empty shell.

Spore Formation.—Spore formation in amebæ takes place usually within 48–72 hours after they are transplanted to fresh medium; but it varies as to time in different species. They first appear as fine, brightly refractive granules, which become larger and larger, by the aggregation of chromidia or by becoming surrounded by a layer of cytoplasm until they may become as large as the nucleus, and in fact have been mistaken by some observers for multiple nuclei. The spores vary in size from .7 to 2 mikra, and they stain faintly and are more readily decolorized than the amebæ. They are extruded from the ameba in 24–48 hours from the time of their first appearance, but an ameba may become encysted before the spores are extruded which has probably led to the error of supposing that the encysted ameba may form spores. The development of the spore into the ameba is direct; the thickening of the wall described by some does not take place if the conditions of growth are favorable. The spore grows larger and larger, and finally the nucleus and the vacuole make their appearance. The spores probably contain cromatin granules. No sexual process was observed.

Methods of Making Permanent Preparations.—Preparations may be obtained by applying a perfectly cleaned cover-glass to the Petri dish culture, and dropping it at once into fixing fluid, but a better way is to drop a hanging plate culture into the fixing fluid. The hanging plate must in this case have been prepared on a very clean cover-glass, or it must have been made on cover-glasses which have been covered with a very thin layer of egg albumen before the agar is spread on it, otherwise the film is apt to become detached. The stain recommended specially by Walker is Mallory's chloride of iron hematoxylin.*

From various sources Walker obtained 44 different cultures which he separates into 10 distinct species using as criteria the characters of the ameboid, the encysted, and the sporulating stages. A. coli Loesch (man); A. hominis sp. n. (man); A. copayæ sp. n. (guinea-pig); A. musculi sp. n. (house mouse); A. gallopavonis sp. n. (turkey); A. ranæ sp. n. (frog); A. muris Grassi (mouse and rat); A. intestinalis sp. n. (various animals); A. enterica sp. n. (various animals); A. fecalis sp. n. (wide range of hosts).

Ameba coli Loesch has a number of synonyms. It is the organism described by Councilman and Lafleur as A. dysenteriæ and taken by them and many others to be the cause of amebic dysentery.

Ameboid stage, circular in outline when at rest; oblong, ligulate, or irregular when in motion; size 9–20 mikrons when in resting stage; pseudopodium normally single, lobose; ectoplasm, hyaline, apparently only in the pseudopodium; entoplasm finely granular; nucleus circular or oval, plastic, 3–5 mikrons, surrounded by a narrow halo; vacuoles one to several, non-contractile. Encysted stage appearing late and slowly in cultures; cysts circular, 6–9 mikrons; wall single, double contoured, regular; contents finely granular; nucleus not visible. Sporulation not frequent; spores never numerous, spheroidal .7–2 mikrons. Movement rather active. Habitat, intestinal tract of man.

Ameba cobayæ Walker. Ameboid stage circular when at rest, oval, oblong, ligulate, less frequently irregular, when in motion; 15.5–25 mikrons in the resting condition; pseudopodium normally single, lobose; ectoplasm, hyaline extensive; entoplasm coarsely granular; nucleus circular but plastic, surrounded by a hyaline halo, otherwise homogeneous; a single contractile vacuole. Encystment early in cultures; cysts circular or oval; 7.5–14 mikrons; wall single, double contoured regular; contents coarsely granular; nucleus not visible. Sporulation frequent; spores numerous spheroidal, .7–2 mikrons. Movement very active. Habitat, intestinal tract of the guinea-pig.

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* Mallory and Wright. Pathological Technic. 1904.

Ameba hominis Walker. Ameboid stage circular when at rest, oval, obovate or oblong when in motion, 6.5–15.4 mikrons at rest; pseudopodium single lobose; ectoplasm apparently only in the pseudopodium, hyaline; entoplasm finely granular; nucleus circular, surrounded by a narrow halo 2.8–4.3 mikrons; a single contractile vacuole. Encysted stage assumed late and slowly in cultures; cyst circular, 4.6–7.7 mikrons wall single, double contoured, regular; contents finely granular, nucleus not visible. Sporulation very frequent; spores spheroidal, numerous, .3–.8 mikrons. Habitat, intestinal tract of man.

Ameba musculi Walker. Ameboid stage circular in resting stage; oval, oblong, obovate, or slightly irregular when in motion; 7.7–13.9 mikrons when at rest; pseudopodium, single, lobose; ectoplasm occupying about half the ameba, hyaline; entoplasm containing a few coarse granules; nucleus spheroidal but plastic, surrounded by a broad halo, otherwise homogeneous, 2.3–3.1 mikrons; single contractile vacuole. Encysted stage assumed only after a very long time and very slowly in cultures; cysts circular or oval, 6.2–7.7 mikrons; wall single, double contoured, regular; contents granular, nucleus not visible. Sporulation rather infrequent; spores not very numerous, spheroidal, 1–2.3 mikrons. Movement fairly active, amebæ wandering far beyond bacterial growth. Habitat, intestinal tract of house mouse.

Ameba gallopavonis Walker. Ameboid stage circular when at rest, oval oblong or irregular when in motion, 18.5–30.8 mikrons in resting stage; pseudopodium normally single, short blunt; ectoplasm more or less distinct, hyaline; entoplasm granular; nucleus circular, 3.4–5.1 mikrons, surrounded by a medium wide halo; a single contractile vacuole; encysted stage assumed rather slowly in cultures; walls two; inner wall very irregular, as if the ameba had become encysted in the ameboid shape; outer wall fairly regular, separated from the inner wall but touching it at the angles, 12.3–20.8 mikrons. Sporulation occasional; spores few, 5–9 spheroidal, 1.2–1.5 mikrons in diameter. Movement sluggish. Habitat, intestinal tract of the turkey.

Ameba ranæ Walker. Ameboid stage circular when at rest, oval, oblong or irregular when in motion, 10.8–15.4 mikrons when at rest; pseudopodium single, broad; ectoplasm slightly evident in the resting ameba, hyaline; entoplasm containing scattered coarse granules; nucleus circular, 1.5–3 mikrons with a relatively broad halo, otherwise homogeneous; a single contractile vacuole. Encysted stage assumed slowly in cultures; cysts circular in outline, 9.8–14 mikrons; wall single, double contoured, regular; contents granular; sporulation moderately frequently; spores 8–16, spheroidal, 1.2–1.5 mikrons. Movement sluggish. Ameba do not wander from bacterial growth in culture. Habitat, intestinal tract of frog.

Ameba muris Grassi. Ameboid stage circular when at rest, 5.4–8.5 mikrons, oval, oblong, occasionally slightly irregular when in motion; pseudopodium, single lobose; ectoplasm and entoplasm scarcely differentiated; hyaline with fine, scattered granules; nucleus very small, spheroidal, with a narrow halo. Encysted stage late, cysts circular, 3.9–5.4 mikrons; wall single, double, contoured, regular, concentrically separated from the contents in old cysts; contents finely granular. Sporulation rather late; spores minute, spheroidal. Movement sluggish; amebæ do not wander from bacterial growth. Habitat, intestinal tract of mouse.

Ameba intestinalis Walker. Ameboid stage circular when at rest; oval, oblong, or less often irregular when in motion; size 9–26 mikrons when at rest;

pseudopodium single, short, broad; ectoplasm scarcely apparent except in pseudopodia, hyaline, entoplasm coarsely granular, frequently vacuolated; nucleus circular, but somewhat plastic, surrounded by a broad halo, otherwise homogeneous, 3.1–6.2 mikrons in diameter; a single contractile vacuole. Encysted stage assumed early, and rapidly; cysts irregular from the beginning walls two; inner wall irregularly polygonal, scalloped or ovoid; outer wall wrinkled and touching in the inner wall at its angles; contents of cysts coarsely granular, nucleus frequently visible. Sporulation moderately frequent; spores numerous, spheroidal, .5–1.5 mikrons. Movement not very active, but the amebæ wander widely over the culture medium. Habitat, intestinal tract of the horse, pig, cat, turkey and perhaps other animals.

Ameba enterica Walker. Ameboid stage circular when at rest, oblong or irregular when in motion; 9.2–15.4 mikrons in diameter when at rest; pseudopodium normally single, blunt, narrow; ectoplasm apparent only in the pseudopodia, hyaline; entoplasm finely granular; nucleus circular in outline, 3.1–6.2 mikrons, surrounded by a broad halo, otherwise homogeneous; a single contractile vacuole. Encysted stage assumed late and slowly; cysts at first circular, later slightly irregular; walls two; inner wall circular, double contoured, at first circular, later becoming polygonal, or lobed in outline; contents finely granular, nucleus not visible; sporulation rather frequent; spores numerous, spheroidal, .8–2 mikrons. Motility moderately active. Habitat, intestinal tract of the rabbit, cat, rat, mouse, turkey, and perhaps other animals.

Ameba fecalis Walker. Ameboid stage circular when at rest, oval, oblong or irregular when in motion, 6.2–15.4 mikrons; pseudopodium normally single, lobose; ectoplasm and entoplasm not differentiated, finely granular, frequently vacuolated, feebly refractive; nucleus small spheroidal, surrounded by a narrow halo, a single contractile vacuole. Encysted stage assumed rather early in cultures; cysts circular or slightly irregular, 3.1–7.8 mikrons; wall single, impervious to stain; contents regularly contract from one or two segments of the wall. Sporulation rather infrequent; spores minute. Movement slow. Habitat, intestinal tract of various vertebrate animals, and probably also free.

Other amebæ have been described in connection with various diseases of animals, but owing to lack of exhaustive descriptions these could not be brought under the classifications of Walker given above, though doubtless some of them in reality belong to one or the other of the species given above. *Ameba dysenteriæ* of Councilman and Lafleur *dysenterica*, Kartulis, *coli felis* Quinke and Roos, *coli mitis* ebenda, *intestini vulgaris* ebenda, *lobosa coli* Celli e Fiocca, *entameba hominis* Casagrandi e Babagallo, *coli* Schaudinn are all regarded as identical with *Ameba coli* Loesch. Unidentified or identified with reserve are *ameba bovis* Liebetanz, *bucallis* Sternberg, *bentali* Grassi, *gingivalis* Gross, *kartulisi* Doflein, *miurai* Ijima, *pulmonalis* Arlault, *ranarum* Grassi, *urogenitalis* Baelz, *buccalis* Prowazek, *undulans* Csatellanil, *gemmipara* Schaudinn.

The Malarial Parasite* (Plasmodium or Hæmatozoön Malariæ).—The organisms of malaria consist of at least three different species, each associated with one of the three types of malarial fever: The *tertian* parasite with benign tertian malarial fever, the parasite reaching maturity in forty-eight

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*See Thayer and Hewetson. The Malarial Fevers of Baltimore. *Johns Hopkins Hospital Reports*. Vol. V. 1895. Thayer. Lectures on the Malarial Fevers. New York. 1897.

hours; the *quartan* parasite with benign quartan malarial fever, the cycle of development requiring seventy-two hours; and the *estivo-autumnal* parasite with malignant, estivo-autumnal fever, developing to maturity in a variable period of from twenty-four to forty-eight hours. The parasites are studied to best advantage in a drop of fresh, fluid blood placed between a cover-glass and slide and examined with an oil-immersion objective. For method of making and staining dry preparations see pages 45 and 108.

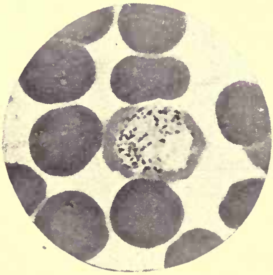

FIG. 106.

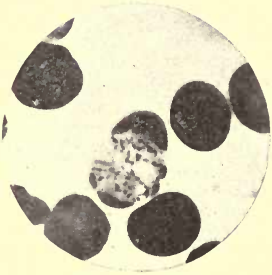

FIG. 107.

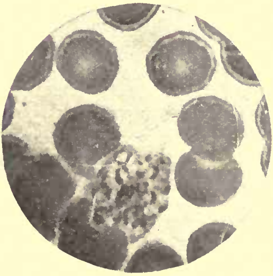

FIG. 108.

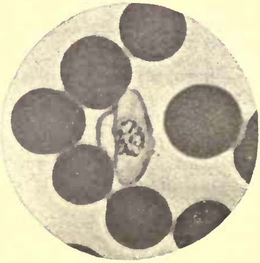

FIG. 109

FIGS. 106–109.—Malarial parasites in various stages. (× 1000.) Figs. 106, 107 and 108 are tertian parasites. Fig. 108 shows the completion of segmentation. Fig. 109 is the crescentic form of the estivo-autumnal parasite.

Tertian Parasite.—This appears in its youngest form as a small, round, colorless, hyaline body within the red corpuscle, seen during and just after the chill of the disease. This body may be actively ameboid, suddenly changing its contour into various forms. Its size gradually increases, and fine, dark, actively motile, dancing pigment granules begin to appear at its periphery.

The red corpuscle harboring the parasite, with the growth of the latter, becomes gradually paler and expands in size. The parasite as it grows loses its earlier ameboid movement, and the pigment granules, still actively motile, accumulate. Near the end of forty-eight hours the organism finally fills the red corpuscle, only a faint rim indicating the latter. The ripe parasite now divides it into from fifteen to twenty-five small, round, hyaline spores, which are arranged somewhat radially about the pigment granules which have lost their motility and become concentrated in a clump at the center of the spore-forming organism. The spores finally break apart and scatter, each destined to invade a red corpuscle and start anew the cycle of development. This cycle may be repeated over and over again, producing a corresponding number of malarial paroxysms.

Certain full-grown parasites do not complete the cycle of development by sporulation, as described, but, breaking loose from the corpuscle, remain as "*extracellular*" bodies. These are seen chiefly after the paroxysm as large, round, pale bodies containing numerous dancing pigment granules scattered through their substance. They ultimately degenerate and disappear. Some of these extracellular forms may be seen to develop long slender processes, flagella, having a very active whip-like motion. Flagella are never observed in perfectly fresh blood, but develop only after the blood has been drawn some time, usually fifteen or twenty minutes.

The extracellular forms of the parasite, the *gametes*, incapable of further development in their human *intermediate host*, can continue their life cycle only when, by chance, they happen to be sucked into the body of a mosquito of the genus Anopheles, the *definite host*, in which they undergo a second complete sexual cycle of development with the ultimate production of spores or sporozoïds. When in turn the spores chance to be inoculated into the blood of man by the bite of an infected Anopheles, the man becomes infected, and the cycle of development in the red corpuscle, already outlined, commences, The second or sexual cycle of the parasite in the mosquito, here described for the tertian organism, applies as well to the other varieties of the malarial organism, namely the quartan and the estivo-autumnal forms, in the case of each starting from the extracellular mature forms of the organism found in the blood of the human host.*

Quartan Parasite.—This resembles quite closely the tertian parasite, but differs from it in certain respects. The young, hyaline, intracorpuscular parasite is more highly refractive, its ameboid motion is less marked and more sluggish, and the pigment granules are darker, much coarser, and have very slight motility. The infected red corpuscles are usually somewhat contracted instead of swollen, and their color is apt to be darker, assuming a bronzed hue. The full-grown parasite is much smaller than the corresponding form of the tertian, approximating the size of a normal red corpuscle. As segmentation begins, a characteristic appearance develops which distinguishes the quartan organism, namely, the coarse pigment granules are drawn toward the center of the parasite in certain converging straight paths, giving a stellate arrangement to the pigment, until finally it becomes clumped entirely at the center in a solid mass. The segmenting forms of the quartan parasite thus present a more symmetrical arrangement of the spores, which often resemble the petals of a "marguerite." These spores are oval and number only from six to twelve, being fewer than those of the tertian segmenting parasite. The quartan extracellular forms are smaller than those of the tertian, being about the size of a red corpuscle, and contain coarse pigment granules in active motility until degeneration occurs. Flagella may develop from certain extracellular forms. The entire development of the quartan parasite occupies about seventy-two hours.

Estivo-autumnal Parasite.—This parasite develops to maturity in from twenty-four to forty-eight hours, and is usually regarded as representing a single species, though certain observers claim to distinguish two distinct varieties. The usual description of a single variety is here adopted. The youngest

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*Lyon. The Inoculation of Malaria by the Mosquito. A Review of the Literature. *Medical Record.* February 17, 1900.

forms (hyaline bodies) resemble those of the tertian and quartan organisms, but are distinctly smaller and more highly refractive. They often present a ring-like appearance. They are ameboid. Pigment granules later appear at their periphery, but are exceedingly minute and scanty, seldom more than one or two being seen. These granules have little or no motility, and in fact are with difficulty made out. The hyaline bodies remain small, seldom exceeding one-third the diameter of a red corpuscle. The infected corpuscle is apt to be crenated, shrunken and dark. These are the forms seen in the circulating blood in early infections; the mature forms, with the exception of the extracellular forms, developing in the spleen and bone-marrow, rarely reach the general circulation. Blood from the spleen shows the full-grown forms in abundance. The segmenting forms resemble those of the tertian parasite both in the numbers of the segments and in their arrangement but are much smaller in the aggregate, as well as in the individual segments.

After the fever has lasted about one week, extracellular forms make their appearance in the circulating blood. These are crescentic, ovoid or small round bodies, containing coarse pigment granules at their center, generally arranged in a ring. The crescents and ovoid bodies are highly refractive and are in length about equal to the diameter of a red corpuscle, sometimes larger. The round forms are smaller than a red corpuscle, with the pigment arranged centrally in a ring. They may become flagellated after the blood has remained outside the body for some minutes. Any of the extracellular bodies may show remnants of the red corpuscle attached to its side, like a bib. The extracellular forms are concerned in the cycle of development of the organism in the mosquito, and are sterile in the human body. They are exceedingly resistant to quinine and may continue in the blood for long periods of time.

Melaniferous leukocytes are seen in the blood, being especially abundant after the paroxysm in all forms of malarial infection.* These are phagocytes which have taken up the pigment granules liberated by the disintegration of the erythrocytes.

Small-pox and Vaccinia.—Micrococci of various sort have been found in the pustules of small-pox and vaccinia, but indicate only a secondary infection. Other microörganisms have been described. The most important are certain bodies often considered protozoa. In both small-box and vaccinia small, round homogeneous bodies, 2 to 4 μ in diameter, have been found in the epithelial cells of the vesicles. Inoculation of vaccine lymph into the rabbit's cornea leads to the production of similar bodies in the epithelial cells of the

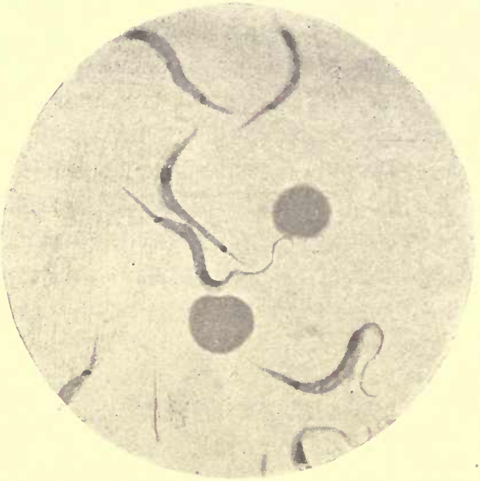

FIG. 110.—Trypanosomes in the blood of the rat. Romanowsky stain. (× 1000.)

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*See also Ewing. *Journal Experimental Medicine.* Vols. V. and VI.

cornea. W. Reed* found small ameboid bodies in the blood in cases of small-pox and vaccinia. Vaccine virus that has been filtered through the Chamberland or Berkefeld filter is still active, but somewhat diminished in power.† From this it may be presumed that the organism causing it is not too small to be seen with the microscope.

Councilman, Magrath and Brinkerhoff,‡ as a result of recent studies, believe that the bodies above mentioned are protozoa. Segmentation of the bodies is described, resulting in the formation of spore-like bodies. The spore-like bodies undergo a further or second cycle of development within the nucleus. The second cycle also ends in segmentation. The two cycles were seen in small-pox; in vaccinia, only the first or extranuclear bodies were observed.

Guarnieri§ was the first to describe these bodies with accuracy, and give to them the name *cytoryctes variolæ.* He found them by staining with carmine, hematoxylin, and safranin in the deeper layers of the epithelium in cases of vaccinia and small-pox. Much opposition to Guarnieri's interpretation of this observation was aroused, but on the other hand, in the epicrisis to a series of articles on small-pox and vaccinia by himself, Magrath, Brinkerhoff, Tyzzer, Southard, Thompson, Bancroft, and Calkins.|| Councilman strongly supports the view that the Guarnieri bodies are living organisms and the cause of the diseases. He believes both small-pox and vaccinia to be caused by the same parasite, and that while certain form of cell degeneration may closely simulate Guarnieri bodies that they are not identical with these.

**Journal Experimental Medicine.* Vol. II. p. 515. See also Anna Williams and Flournoy, and W. H. Park. *New York University Bulletin Medical Sciences.* Vol. II. October, 1902.

†Negri. *Zeitschr. f. Hygiene.* Vol. LIV., No. 3. Oct. 26, 1906. p. 327. Also see *Bull. de l'Inst. Past.* Vol. V., No. 1. Jan. 15, 1907. p. 23.

‡*Journal Medical Research.* Vol. IX. May, 1903. *Ibid.* Vol. XI.

§*Arch. per le Sci. Med.* XXVI. p. 403; *Centralblt. f. Bakterio.* XVI. p. 299.

||*Journ. Med. Research.* Vol. II., No. 1. pp. 7-361.

Ewing* on the contrary comes to the conclusion from his study of the subject, in which he corroborates and extends the observations of others, that the bodies are probably degenerated tissue cells. The fact that the forms met with are peculiar to variola and to vaccinia, not found in other morbid processes nor in healthy tissue, Ewing finds is no convincing proof of the organized character of the bodies; for the forms of cell degeneration found in diphtheria, in measles, in glanders, in rabies, are all peculiar and characteristic in these diseases. No other agent has been found which will cause the same form of cell degeneration which is caused by the toxin of diphtheria, and this is true in regard to other diseases than diphtheria.

It is thus evident that competent authorities are not as yet agreed upon the nature of the organism causing small-pox. Still it seems settled that there are characteristic bodies which are always found in the disease whether these be living parasites or merely peculiar forms of cell degeneration. These bodies are also found in vaccinia, and in the lesions produced by the inoculation of monkeys, and also in the lesions of the cornea of rabbits which have inoculated in the eye with small-pox or vaccine virus.

SCARLET FEVER AND MEASLES.

Field† obtained in specimens from the skin taken at autopsy and in the serum secured intravitam by the use of a small blister-plaster in cases of scarlet fever and measles preparations showing protozoön-like bodies previously described by Mallory‡ in material secured at autopsy in cases of scarlet fever only. Field used many different kinds of stains, and found Giemsa to give the best results; Hastings almost as good. The bodies which are of interest resemble closely the extracellular forms of the malarial parasite. They show a pale blue protoplasm with one or more granules which vary in size from a mere point to a particle occupying almost the total diameter of the body. In four cases they were arranged so as to imitate the malarial rosettes. Field states in regard to the character of these bodies, that while it cannot be stated that they are not protozoa the majority of them arise from degenerating cells. The bodies in the blister-fluid resemble other granular bodies seen in the blood under certain con-

---

*Ewing. *Journ. Med. Research.* Vol. XII. 1904. p. 509. Vol. XIII. 1905. pp. 233–252.

†*Journ. Exper. Med.* Vol. VII. Feb.–Nov. 1905. pp. 343–350.

‡*Journ. Med. Research.* Vol. X. 1904. p. 483.

ditions, and also in vaccine lymph, and in emulsions from tissues and in exudates. He therefore regards them as for the most part, if not wholly, products of degenerating tissue cells and of leukocytes.

YELLOW FEVER.

It has already been indicated (page 149) that the study of cases of yellow fever has failed to prove that this disease is caused by bacteria. On the other hand, evidence that it is transmitted by the mosquito, Stegomyia, has been increasing.

Piroplasma Bigeminum, Pirosoma Bigeminum, Apiosoma Bigeminum.—Theobald Smith was the first to describe this parasite which is found in the blood of cattle suffering with cattle fever, though Babes reported having found certain cell inclusions in the blood of sick cattle in Roumania, and Smith and Kilborn made extensive studies of the organism. It is pear-shaped occurring in pairs with the pointed end toward each other, each half of the pair is 2 to 4 mikrons long, by 1½ to 2 mikrons wide. It occurs inside the red corpuscles. Pseudopodia are described by Nuttall and Graham-Smith.* It stains well with the basic dyes and is well brought out by Romanowsky. It has never yet been cultivated. It is spread from sick to diseased animal by a tick, *Boöphilus bovis sive annulatus*, in America, by several species of ticks apparently in Germany. The ticks seem to serve merely as carriers, the organism appears to undergo no development in the body of the tick. Other animals than cattle also suffer from infection with organisms of similar description. Sheep, dogs, horses, and apes have been found to harbor a parasite of this character. It has been stated that men have also been found with a similar organism in the blood in cases of "Spotted fever" in Montana as reported by Wilson and Chowning.† But Stiles‡ failed to corroborate this statement. Ricketts§ found that the disease is transmitted by a tick, *Dermacentor*

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*[*Journ. Hygiene.*] Vol. VI. 1906. p. 586.

†Rep. Montana State Board of Health. 1901–2. Also *Journ. Infect. Diseases.* Vol. I., No. 1. Jan. 1, 1904. pp. 31–57.

‡Pub. Health and Mar. Hosp. Bull. No. 20, 1905.

§*Journ. Infect. Dis.* Vol. IV, No. 1. Jan. 1, 1907. pp. 141–153.

occidentalis, both male and female. He also found that one attack affords a high degree of immunity. The virus is not filterable through a Berkefeld filter. The parasite, whatever it may be, is found in the body fluids generally. The infectiousness of the blood is largely destroyed by grinding the infectious blood in a ball-mill.

Certain peculiar bodies suggestive of parasites have been found in the lesions of a tropical disease known as "Delhi Boil." Wright* gives in substance the following description of the disease and the bodies alluded to. The disease resembles some of the manifestations of syphilis and tuberculosis, and is held to be infectious. It consists of multiple nodules in the skin which finally ulcerate. It lasts for months or for a year or longer. In smear preparations made from material obtained from one of the lesions, fixed in methyl alcohol, and stained by Wright's modification of the Romanowsky a large number of the bodies first described by Cunningham and believed by him to be living parasites were observed. They were for the most part round, though other forms were also present, and from 2 to 4 mikrons in diameter. Most of the periphery was robin's egg blue, the center was unstained. Each of the bodies showed a larger and a smaller lilac-colored mass. Sections of the same material showed the same bodies. The evidence adduced as to the parasitic nature of the bodies is insufficient to show that they are not the products of cell degeneration. Animal experiments were negative.

**Journ. Med. Research.* Vol. X., 1904. pp. 472–482.

APPENDIX.

GLOSSARY OF TERMS.*

AGAR HANGING BLOCK, a small block of nutrient agar cut from a poured plate, and placed on a cover-glass, the surface next the glass having been first touched with a loop from a young fluid culture or with a dilution from the same. It is examined upside down, the same as a hanging drop.

AMEBOID, assuming various shapes like an ameba.

AMORPHOUS, without visible differentiation in structure.

ARBORESCENT, a branched, tree-like growth.

BEADED, in stab or ~~strok~~e, disjointed or semi-confluent colonies along the line of inoculation.

BRIEF, a few days, a week.

BRITTLE, growth dry, friable under the platinum needle.

BULLATE, growth rising in convex prominences, like a blistered surface.

BUTYROUS, growth of a butter-like consistency.

CHAINS,

Short chains, composed of 2 to 8 elements.

Long chains, composed of more than 8 elements.

CILIATE, having fine, hair-like extensions, like cilia.

CLOUDY, said of fluid cultures which do not contain pseudozoogloeae.

COAGULATION, the separation of casein from whey in milk. This may take place quickly or slowly, and as the result either of the formation of an acid or of a lab ferment.

CONTOURED, an irregular, smoothly undulating surface, like that of a relief map.

CONVEX, surface the segment of a circle, but flattened.

COPROPHYL, dung bacteria.

CORIACEOUS, growth tough, leathery, not yielding to the platinum needle.

CRATERIFORM, round, depressed, due to the liquefaction of the medium.

CRETACEOUS, growth opaque and white, chalky.

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* Chart of Society of American Bacteriologists. Jan., 1908.

CURLED, composed of parallel chains in wavy strands, as in anthrax colonies.

DIASTASIC ACTION, same as **DIASTATIC,** conversion of starch into water-soluble substances by diastase.

ECHINULATE, in agar stroke a growth along line of inoculation, with toothed or pointed margins; in stab cultures growth beset with pointed outgrowths.

EFFUSE, growth thin, veily, unusually spreading.

ENTIRE, smooth, having a margin destitute of teeth or notches.

EROSE, border irregularly toothed.

FILAMENTOUS, growth composed of long, irregularly placed or interwoven filaments.

FILIFORM, in stroke or stab cultures a uniform growth along line of inoculation.

FIMBRIATE, border fringed with slender processes, larger than filaments.

FLOCCOSE, growth composed of short curved chains, variouly oriented.

FLOCCULENT, said of fluids which contain pseudozoogloeae, *i. e.*, small adherent masses of bacteria of various shapes and floating in the culture fluid.

FLUORESCENT, having one color by transmitted light and another by reflected light.

GRAM'S STAIN, a method of differential bleaching after gentian violet, methyl violet, etc. The + mark is to be given only when the bacteria are deep blue or remain blue after counterstaining with Bismark brown.

GRUMOSE, clotted.

INFUNDIBULIFORM, form of a funnel or inverted cone.

IRIDESCENT, like mother-of-pearl. The effect of very thin films.

LACERATE, having the margin cut into irregular segments as if torn.

LOBATE, border deeply undulate producing lobes (see undulate).

LONG, many weeks, or months.

MAXIMUM TEMPERATURE, temperature above which growth does not take place.

MEDIUM, several weeks.

MEMBRANOUS, growth thin, coherent, like a membrane.

MINIMUM TEMPERATURE, temperature below which growth does not take place.

MYCELIOID, colonies having the radiately filamentous appearance of mold colonies.

NAPIFORM, liquefaction with the form of a turnip.

NITROGEN REQUIREMENTS, the necessary nitrogenous food. This is determined by adding to *nitrogen-free* media the nitrogen compound to be tested.

OPALESCENT, resembling the color of an opal.

OPTIMUM TEMPERATURE, temperature at which growth is most rapid.

PELLICLE, in fluid bacterial growth either forming a continuous or an interrupted sheet over the fluid.

PEPTONIZED, said of curds dissolved by trypsin.

PERSISTENT, many weeks or months.

PLUMOSE, a fleecy or feathery growth.

PSEUDOZOOGLOEAE, clumps of bacteria, not dissolving readily in water, arising from imperfect separation, or more or less fusion of the components, but not having the degree of compactness and gelatinization seen in zoogloeae.

PULVINATE, in the form of a cushion, decidedly convex.

PUNCTIFORM, very minute colonies, at the limit of natural vision.

RAISED, growth thick, with abrupt or terraced edges.

RHIZOID, growth of an irregular branched or root-like character, as in *B. mycoides*.

RING, Same as **RIM,** growth at the upper margin of a liquid culture, adhering more or less closely to the glass.

REPAND, wrinkled.

RAPID, Developing in 24 to 48 hours.

SACCATE, liquefaction the shape of an elongated sack, tubular, cylindrical.

SCUM, floating islands of bacteria. an interrupted pellicle or bacterial membrane.

SLOW, requiring 5 or 6 days or more for development.

SHORT, applied to time, a few days, a week.

SPORANGIA, cells containing endospores.

SPREADING, growth extending much beyond the line of inoculation, *i. e.*, several millimeters or more.

STRATIFORM, liquefying to the walls of the tube at the top and then proceeding downwards horizontally.

THERMAL DEATH-POINT, the degree of heat required to kill young fluid cultures of an organism exposed for 10 minutes (in thin-walled test-tubes of a diameter not exceeding 20 mm.) in the thermal water-bath. The water must be kept agitated so that the temperature shall be uniform during the exposure.

TRANSIENT, a few days.

TURBID, cloudy with flocculent particles; cloudy plus flocculence..

UMBONATE, having a button-like, raised center.

UNDULATE, border wavy, with shallow sinuses.

VERRUCOSE, growth wart-like, with wart-like prominences.

VERMIFORM-CONTOURED, growth like a mass of worms, or intestinal coils.

VILLOUS, growth beset with hair-like extensions.

VISCID, growth follows the needle when touched and withdrawn, sediment on shaking rises as a coherent swirl.

ZOOGLOEAE, firm gelatinous masses of bacteria, one of the most typical examples of which is the *Streptococcus mesenterioides* of sugar vats (*Leuconostoc mesenterioides*), the bacterial chains being surrounded by enormously thickened firm covering, inside of which there may be one or many groups of the bacteria.

NOTES.

(1) For decimal system of group numbers see Table I. This will be found useful as a quick method of showing close relationships inside the genus, but is not a sufficient characterization of any organism.

(2) The morphological characters shall be determined and described from growths obtained upon at least one solid medium (nutrient agar) and in at least one liquid medium (nutrient broth). Growths at 37° C shall be in general not older than 24 to 48 hours, and growths at 20° C. not older than 48 to 72 hours. To secure uniformity in cultures, in all cases preliminary cultivation shall be practised as described in the revised Report of the Committee on Standard Methods of the Laboratory Section of the American Public Health Association. 1905.

(3) The observation of cultural and bio-chemical features shall cover a period of at least 15 days and frequently longer, and shall be made according to the revised Standard Methods above referred to. All media shall be made according to the same Standard Methods.

(4) Gelatin stab cultures shall be held for 6 weeks to determine liquefaction.

(5) Ammonia and indol tests shall be made at end of tenth day, nitrite tests at end of fifth day.

(6) Titrate with $\frac{N}{20}$ NaOH, using phenolphthalein as an indicator: make titrations at same times from blank. The difference gives the amount of acid produced.

3. **ENDOSPORES.**
Form, *round, elliptical, elongated.*
Limits of Size....................
Size of Majority ....
Wall, *thick, thin*
Sporangium wall, *adherent, not adherent.*
Germination, *equatorial, oblique, polar, bipolar, by stretching.*

4. **Flagella** No..........Attachment *polar, bipolar, peritrichiate.*
How stained.........................

5. **Capsules,** present on....................

6. **Zoogloea,** Pseudozoogloea.

7. **Involution Forms,** on........in.......days at............° C.

8. **Staining Reactions.**
1:10 watery fuchsin, gentian violet, carbol fuchsin,
Loeffler's alkaline methylene blue.
Special Stains
Gram..........................Glycogen..................
Fat ....Acid fast...................
Neisser......................

II. CULTURAL FEATURES (²)

1. **Agar Stroke.**
Growth, *invisible, scanty, moderate, abundant.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*
Optical Characters, *opaque, translucent, opalescent, iridescent.*
Chromogenesis (⁸) ....
Odor, *absent, decided, resembling*.......................
Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*
Medium, *grayed, browned, reddened, blued, greened.*

2. **Pototo.**
Growth *scanty, moderate, abundant, transient, persistent.*
Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
Elevation of growth, *flat, effuse, raised, convex.*
Lustre, *glistening, dull, cretaceous.*
Topography, *smooth, contoured, rugose, verrucose.*

Chromogenesis (8).................. Pigment in water *insoluble, soluble;* other solvents..................................

Odor, *absent, decided, resembling*...............................

Consistency, *slimy, butyrous, viscid, membraneous, coriaceous, brittle.*

Medium, *grayed, browned, reddened, blued, greened.*

3. **Loeffler's Blood-serum.**

Stroke *invisible, scanty, moderate, abundant.* Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*

Elevation of growth *flat, effuse, raised, convex.*

Lustre, *glistening, dull, cretaceous.*

Topography, *smooth, contoured, rugose, verrucose.*

Chromogenesis (8)..................

Medium *grayed, browned, reddened, blued, greened.*

Liquefaction begins in..........d, complete in..........d.

4. **Agar Stab.**

Growth *uniform, best at top, best at bottom; surface growth scanty, abundant; restricted, wide-spread.*

Line of puncture, *filiform, beaded, papillate, villous. plumose, arborescent; liquefaction.*

5. **Gelatin Stab.**

Growth *uniform, best at top, best at bottom.*

Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent.*

Liquefaction, *crateriform, napiform, infundibuliform, saccate, stratiform;* begins in ....d, complete in ....d.

Medium *fluorescent, browned,*....................

6. **Nutrient Broth.**

Surface growth, *ring, pellicle, flocculent, membraneous, none.*

Clouding *slight, moderate, strong; transient, persistent; none; fluid turbid.*

Odor, *absent, decided, resembling*..............................

Sediment, *compact, flocculent, granular, flaky, viscid on agitation, abundant, scant.*

7. **Milk.**

Clearing without coagulation.

Coagulation *prompt, delayed, absent.*

Extrusion of whey begins in...............days.

Coagulum *slowly peptonized, rapidly peptonized.*

Peptonization begins on...........d, complete on..........d.

Reaction, 1d......, 2d........, 4d........, 10d......., 20d...,

Consistency, *slimy, viscid, unchanged.*
Medium *browned, reddened, blued, greened.*
Lab ferment, *present, absent.*

8. **Litmus Milk.**
Acid, alkaline, acid then alkaline, no change.
Prompt reduction, no reduction, partial slow reduction.

9. **Gelatin Colonies.**
Growth *slow, rapid.*
Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.*
Elevation, *flat, effuse, raised, convex, pulvinate, crateriform (liquefying).*
Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, filamentous, floccose, curled.*
Liquefaction, *cup, saucer, spreading.*

10. **Agar Colonies.**
Growth *slow, rapid,* (temperature................)
Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.*
Surface *smooth, rough, concentrically ringed, radiate, striate.*
Elevation, *flat, effuse, raised, convex, pulvinate umbonate.*
Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, floccose curled.*
Internal structure, *amorphous, finely-, coarsely-granular, grumose, filamentous, floccose, curled.*

11. **Starch Jelly.**
Growth, *scanty, copious.*
Diastasic action, *absent, feeble, profound.*
Medium stained..................

12. **Silicate Jelly (Fermi's Solution).**
Growth *copious, scanty, absent.*
Medium stained..................

13. **Cohn's Solution.**
Growth *copious, scanty, absent.*
Medium *fluorescent, non-fluorescent.*

14. **Uschinsky's Solution.**
Growth *copious, scanty, absent.*
Fluid *viscid, not viscid.*

15. **Sodium Chloride in Bouillon.**
Per cent. inhibiting growth........................

16. **Growth in Bouillon over Chloroform,** *unrestrained, feeble, absent.*

17. **Nitrogen.** Obtained from *peptone, asparagin, glycocoll, urea, ammonia salts, nitrogen.*

18. **Best media for long-continued growth** ....
....

19. **Quick tests for differential purposes** ....
....
....

III. PHYSICAL AND BIOCHEMICAL FEATURES.

1. Fermentation-Tubes containing peptone-water or Sugar-free bouillion and	Dextrose	Saccharose	Lactose	Maltose	Glycerin	Mannit				
Gas production, in per cent.										
$\left(\frac{H}{CO_2}\right)$										
Growth in closed arm										
Amount of acid produced 1d.										
Amount of acid produced 2d.										
Amount of acid produced 4d.										

2. **Ammonia production,** *feeble, moderate, strong, absent, masked by acids.*

3. **Nitrates in nitrate broth.**
Reduced, not reduced.
Presence of nitrites................ammonia................
Presence of nitrates..................free nitrogen..........

4. **Indol production,** *feeble, moderate, strong.*

5. **Toleration of Acids:** *great, medium, slight.*
Acids tested.......................

6. **Toleration of Na O H:** *great, medium, slight.*

7. **Optimum reaction for growth in bouillon,** stated in terms of Fuller's scale............................

8. **Vitality on culture media:** *brief, moderate, long.*

9. **Temperature relations:**
Thermal death-point (10 minutes exposure in nutrient broth when this adapted to growth of organism).............C.
Optimum temperature for growth.........C.; or best growth at 15° C., 20° C., 25° C., 30° C., 37° C., 40° C., 50° C., 60° C.
Maximum temperature for growth................C.
Minimum temperature for growth..............C.

10. **Killed readily by drying:** resistant to drying.

11. **Per cent.** killed by freezing (salt and crushed ice or liquid air) .....

12. **Sunlight:** Exposure on ice in thinly sown agar plates: one-half plate covered (time 15 minutes), *sensitive, not sensitive.*
Per cent. killed.......................

13. **Acidsproduced**......................
14. **Alkalies produced**....................
15. **Alcohols**...........................
16. **Ferments**; *pepsin, trypsin, diastase, invertase, pectase, cytase, tyrosinase, oxidase, peroxidase, lipase, catalase, glucase, galactase, lab.* etc...............................

....

17. **Crystals formed**:...
18. **Effect of germicides**:

Substance	Method used	Minutes	Temperature	Killing quantity	Amt. required to restrain growth

IV. PATHOGENICITY.

1. **Pathogenic to Animals.** *Insects, crustaceans, fishes, reptiles, birds, mice, rats, guinea-pigs, rabbits, dogs, cats, sheep, goats, cattle, horses, monkeys, man*.......
2. **Pathogenic to Plants**:

....

....

....

3. **Toxins**, *soluble, endotoxins.*
4. **Non-toxin forming.**
5. **Immunity bactericidal.**
6. **Immunity non-bactericidal.**
7. **Loss of virulence on culture media**: *prompt, gradual, not observed* in ....months.

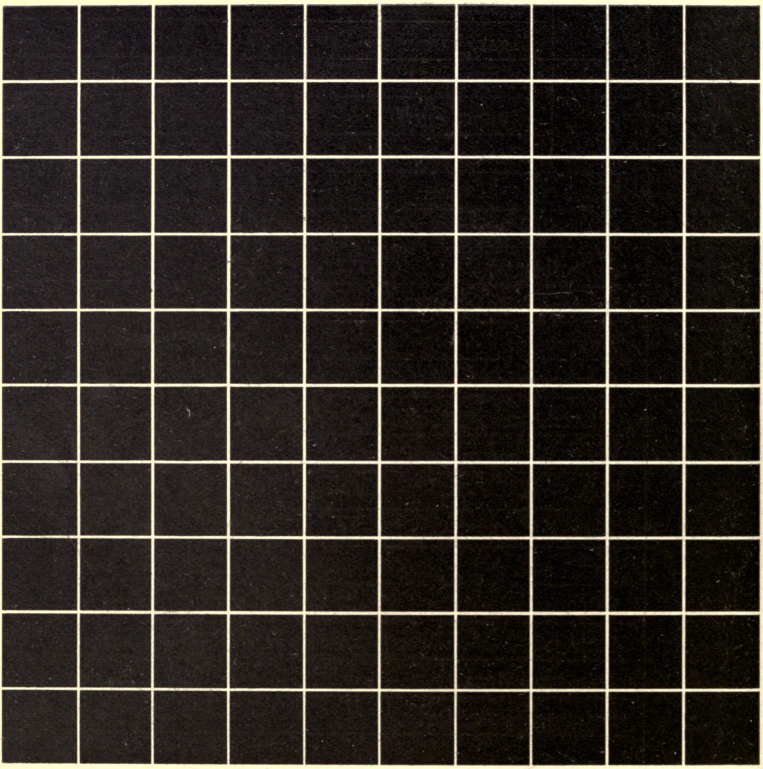
BRIEF CHARACTERIZATION.

Mark + or O, and when two terms occur on a line erase the one which does not apply unless both apply.

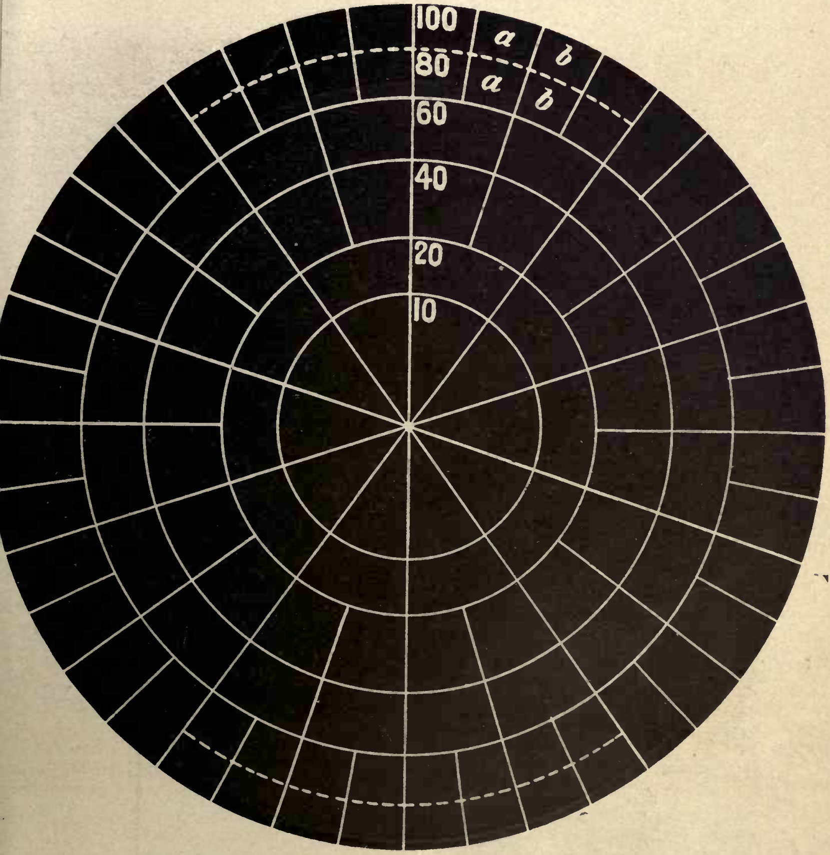
(2) MORPHOLOGY		Diameter over 1 μ.	
		Chains, filaments	
		Endospores	
		Capsules	
		Zooglœa, Pseudozooglœa	
		Motile	
		Involution forms	
		Gram's Stain	
(3) CULTURAL FEATURES	Broth	Cloudy, turbid	
		Ring	
		Pellicle	
		Sediment	
	Agar	Shining	
		Dull	
		Wrinkled	
		Chromogenic	
	Gel. Plate	Round	
		Proteus-like	
		Rhizoid	
		Filamentous	
		Curled	
	Gel. Stab	Surface-growth	
		Needle-growth	
	Potato	Moderate, absent	
		Abundant	
		Discolored	
		Starch destroyed	
		Grows at 37° C.	
		Grows in Cohn's Sol.	
		Grows in Uschinsky's Sol.	

BRIEF CHARACTERIZATION.—*Continued.*

BIOCHEMICAL FEATURES	Liquefaction	Gelatin (4)	
		Blood-serum	
		Casein	
		Agar, mannan	
	Milk	Acid curd	
		Rennet curd	
		Casein peptonized.	
		Indol (5)	
		Hydrogen sulphide	
		Ammonia (5)	
		Nitrates reduced (5)	
		Fluorescent	
		Luminous	
DISTRIBUTION		Animal pathogen, epizoon	
		Plant pathogen, epithyte	
		Soil	
		Milk	
		Fresh water	
		Salt water	
		Sewage	
		Iron bacterium	
		Sulphur bacterium	



Surface divided in square centimeters for counting colonies.


100
80
60
40
20
10
a
b
a
b

Jeffer's plate (Bausch and Lomb). For counting colonies of bacteria on circular plates. The area of each division is one square centimeter.

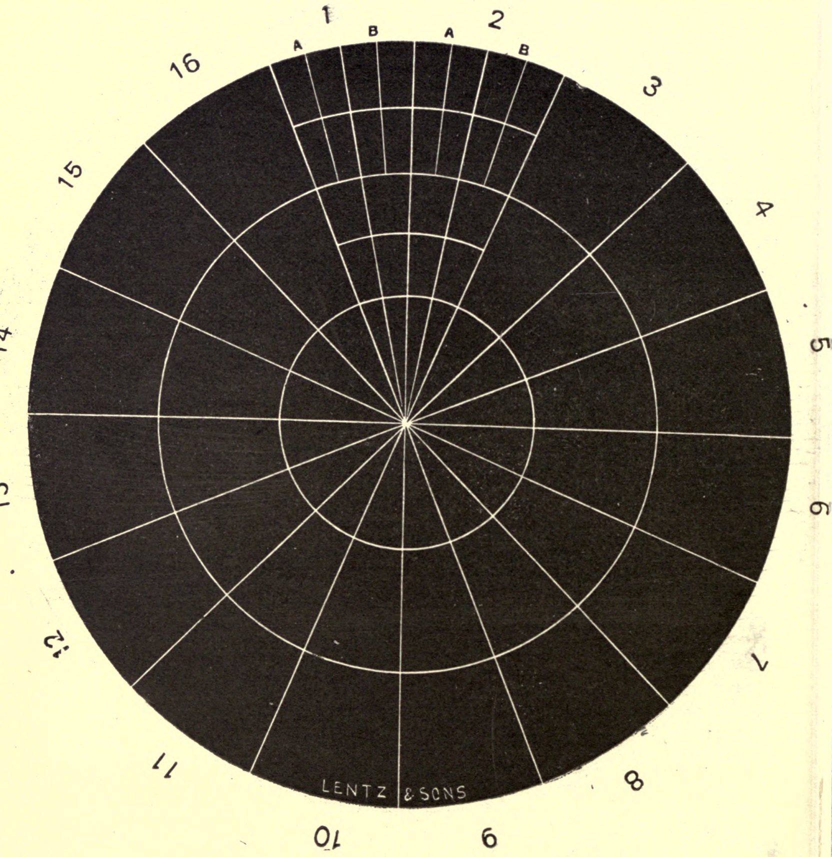

1
2
A
B
A
B
3
4
5
6
7
8
9
10
11
12
14
15
16
LENTZ & SONS

Plate for counting colonies of bacteria in Petri dishes.

INDEX.

Abbé condenser, 20
Abrin compared with bacterial toxins, 189
  immunity from, acquired by injections of, 209
Abscesses, bacteria which cause, 284
  pathological process involved, 282
  metastatic, caused by S. pyogenes aureus, 290
    pathological process involved, 290
Absorbent cotton for plugging test-tubes, 76
  saturated with pyrogallic acid for anaerobic cultures, 91
Accidental infection of laboratory workers, 115
Acid, acetic, for decolorizing background of cover-glass preparations, 29
      for tissues, 41
    as product of growth of bacteria, 131
    in Welch's capsule stain, 49
  alcohol for decolorizing after stains for tubercle bacilli, 35
  aniline dyes which are not suitable for bacterial stains, 28, 35
  boric as an antiseptic, 257
  butyric, formation of by B. butyricus, 270
    by various bacteria, 131
  carbolic, as a germicide, 250
    as end product in the growth of bacteria, 129
    germicidal power enhanced by hydrochloric acid, 251
      neutralized by sodium sulphate, 245
      neutralized by lime, 251
    in Ziehl's carbol-fuchsin stain, 34
Acid, formic, as a product of bacterial growth, 131
  fuchsin not suitable for bacterial stains, 28, 35
  hydrochloric, with carbolic acid as germicide, 251
    with corrosive sublimate for disinfecting purposes, 249
    as germicide in gastric juice, 164
  hydrochloric-alcohol as decolorizer, 35
  lactic, as product of bacterial growth, 131
    in sour milk, 157
    formed by B. acidici lactici and other bacteria of the same group, 131
  picric, as contrast stain for tissues, 28
  propionic, formed by bacterial growth, 131
  pyrogalic, for cultivating anaerobes, 91
  rosolic, for detection of acid formed in cultures, 73
  sulphuric, in Gabbett's decolorizer, 35
    as decolorizer with other stains, 35
Acid-proof bacilli, branching forms in suppurative and necrotic lesions and in bronchopneumonia, 370
  in butter, in cow dung and on grass, 351
  in gangrene of the lung, 351
  in milk, 151
  in smegma preputialis, 33, 163
  resist decolorization with acids, 33
  retain the stain after treatment with Gabbett's solution, 36
Acids, formation of, by bacteria, 131
Acquired immunity, 200

Actinomyces, higher bacteria with branching filaments, 277
  in pus formation, 284
  tubercle bacillus possibly of this group, 287
  as the cause of "lump-jaw" in cattle, actinomycosis bovis, 366
Actinomycosis bovis, or "lump-jaw," in cattle, 366
Active immunity, 211
Acute miliary tuberculosis, 358
Adherence of bacteria to moist surfaces, 80
Aerobic bacteria, definition of, 127
Aerobioscope, 138
Agar-agar, azolitmin, 71
  glycerin, 70
  dextrose, 71
  lactose, 71
  litmus, 71
  neutral-red, 71
  plain, 69
  saccharose, 71
Age, relation of, to infection, 179
Agglutinating substances in blood-serum, 190
Agglutinins, definition, 190
  development of, in blood, 191
  group, 190
  specific, 191
Aggressins, 217
Air, bacteria of, 137
  B. anthracis in, 137
  effect of altitude on number of bacteria in, 137
  method of examination, 137
  pathogenic bacteria conveyed by, 175
    bacteria on floating particles of sputum in, 137
    conditions affecting number of bacteria in, 137
    species of bacteria in, 137
  in veins found after death due to fermentation, 6, 268
Albumen, culture media containing, 75
  glycerin fixative for tissues (Mayer), 40
  specific precipitins for, 196
Alcohol, acid for decolorizing, after staining bacteria with carbol fuchsin, 35
  fixation of tissues with, 37
  in Gram's stain, 31
  in Ziehl's stain, 34
Alcohol, relation to infection when used as beverage, 179
Alexins, 226
Alimentary canal, bacteria of, 164
Alum as a coagulant in water filtration, 141
Amboceptor, 223, 230
Ameba beutali, 417
  bovis, 417
  buccalis, 417
  cobayæ, 415
  coli, 415
  coli felis, 417
  coli mitis, 417
  dysenterica, 417
  entameba hominis, 417
  enterica, 417
  fecalis, 417
  gallopavonis, 416
  gemmipara, 417
  gingivalis, 417
  hominis, 416
  intestinalis, 416
  intestini vulgaris, 417
  Kartulis, 417
  lobosa coli, 417
  miurai, 417
  muris, 416
  musculi, 416
  pulmonalis, 417
  ranæ, 416
  ranarum, 417
  undulans, 417
  urogenitalis, 417
Amebæ, cultivation of, 411
    hanging-plate method, 413
  isolation of, 412
  permanent preparations of, 415
  reproduction in, 413
  sporeformation, 414
American filtration system, 141
American Public Health Association, directions for preparation of culture media, 66
Ammonia, conversion of, into nitrous acid, 131
Anaerobic bacteria, cultivation of, 90
  definition of, 127
Anaphylaxis, 211
Anilin dyes, acid unsuited for staining bacteria, 28
    alkaline, for staining bacteria, 28
    alcoholic solutions for preparation of aqueous solutions, 28

Anilin dyes, aqueous solutions for staining bacteria, 28
  as germicides, 251
  oil, aqueous solution of, for Gram's stain, 30, 42
   for Weigert's stain, 30, 42
Animals, autopsies on, 104
  care of, 104
  inoculation of, as means of obtaining pure cultures, 94
   for testing pathogenic properties of bacteria, 102
Anopheles as conveyers of malaria, 177
  as hosts in which the malarial parasite develops, 418
Anthrax bacillus (see B. anthracis)
  symptomatic, or "black-leg," of cattle, virus for, 202
Antiagglutinins, 199
Antibodies, 199
Antilysins analogy to antitoxins, 199
  combining affinities with complement and immune body, 228
Antiprecipitins analogy with antitoxin, 199
Antiseptic, definition of, 238
Antitoxic unit in diphtheria, 348
Antitoxins analogy with other antibodies, 199
  behavior of mixtures of, with toxin, 223
  for diphtheria, principle involved in the production, 209
  for tetanus, 331
   method employed in the production, 346
Antitoxin-toxin mixtures, peculiar behavior of, 223
Argentamin as germicide, 250
Argonin as germicide, 250
Argyrole as germicide, 250
Arnold's steam sterilizer, 55
Arrhenius, on analogy between antitoxin-toxin mixtures and esters, 226
Arrow-poison, tetanus bacillus in, 6, 22
Arthritis, pyogenic cocci in, 290
  micrococcus lanceolatus in, 306
  gonococcus in, 314
Arthrospores, 123
Artificial cultivation of bacteria, effect of, on virulence, 82
Asiatic cholera, 396
Aspergillus glaucus, 279
Autoclave, 60
Auto-infection, 177
Autopsies on animals, precautions against accidental infection at, 104
   cultures and cover-glass preparations from the organs, 105
   technic employed, 104
  on human cadavers, 109
Avenues of entrance of bacteria into the body, 172
Avian tuberculosis, 362

Babes-Ernst bodies, 121
Bacilli, branching forms rarely encountered, 119
  acid-proof, in butter and on grass, 351
   in gangrene of the lung, 351
   in milk, 151
   in smegma preputialis, 33, 163
   retain the stain after treatment with Gabbett's solution, 36
   tubercle bacilli, 36
   tubercle, leprosy and other bacilli, 33
Bacillus acidi lactici, Hueppe, 273
  acidophilus, 165
  lactis aerogenes in upper part of intestines of children, 164
   morphology and cultural characters of, 386
  aerogenes capsulatus, 325
  amylobacter in the stomachs of ruminants, 270
   description of, 270
  anthracis, cultivation in large amounts for extraction of endotoxin susceptible animals, 333
   early observations of, in the blood of animals, 14
   formation of spores in oxygen, 332
   in air, 137
   infection of laboratory workers, 334
   in soil, 135
   in "wool-sorter's" disease, 137, 334
   morphology, cultural characters, pathogenic properties, 333

Bacillus anthracis, predisposing causes in immune animals, 179
  rapidity of distribution in the animal on inoculation, 174
    resistance of spores to carbolic acid, 332
      to corrosive sublimate, 333
      to heat, 332
    spores present in the soil, 135
    "vaccines" against, 334
  bifidus in human feces, 165
  botulinus in decayed meat and other foods, 159
  buccalis maximus, 277
  butyricus, Hueppe, description of, 270
    sive amylobacter, Prasmowski, 270
  capsule of Pfeiffer, 315
  capsulatus septicus, 306
  coli communis in milk, 152, 159
    cystitis due to, 290
    detection of, in water, 146
    differentiation from B. typhosus, 382
    in healthy intestines of man and other animals, 165
    in intestines of infants, 164
    morphology, cultural characters, 381
    peritonitis due to, 288
    pus formation due to, 284
    resemblance to B. pneumoniæ (Friedlander), 315
    resisting power of toxin of, to heat, 190
    significance of, in water, 146
  comma, of Asiatic cholera (see Spirillum choleræ Asiaticæ)
  cyanogenus, 273
  definition of the word, 3
  various forms, 4
  diphtheriæ, accidental infection of laboratory workers, 115
    antitoxin unit, 348
    found on the bed clothing of patient, 174
    morphology, cultural characters, 338, 342
    pathogenic properties for experiment animals, 344
    preparation of the antitoxin, 346
    staining peculiarities, 338
    theories of immunity from, 212
Bacillus diphtheriæ, toxin-antitoxins mixtures, 223
  enteritidis, Gärtner, in decomposed meats and other foods, 159
    related to paracolon bacillus, 386
  erythrosporus, 273
  fluorescens liquefaciens, 269
    putidus, 269
  fusiformis, 275, 349
  icteroides, 171
  Indicus, 269
  influenzæ, 336
  hemorrhagic septicemia (Howard, 316
  Klebs-Löffler (see Bacillus diphtheriæ)
  lepræ, staining reaction like that of tubercle bacilli, 33
    description, morphology, pathogenic properties, 362
  malleic agglutination with homologous serum, 193
    morphology, cultural characters, pathogenic properties, 364
  magaterium, 270
  mesentericus vulgatus (potato bacillus), for class demonstration, 114
    high resisting power of spores, 123
    morphology, cultural characters, 271
  mucosus (Blumer), 316
    capsulatus (see Bacillus Pneumoniæ, Friedländer)
  mycogenes, cultural characters, morphology, pathogenic properties, 317
  mycoides (B. ramosus), 271
  Neopolitanus (probably the same as B. coli communis, *q. v.*)
  œdematis maligni cultural characters, 327
  of blue milk (see Bacillus cyanogenus), 273
    in soil, 135
  of bubonic plague, 320
  of chancroid, 314
  of diphtheria (see Bacillus diphtheriæ)
  of Ducrey (see of chancroid)
  of dysentery (see Bacillus dysenteriæ)

- Bacillus of Eberth (see Bacillus typhi abdominalis)
  - of Emmerich (see Bacillus coli communis)
  - of Escherich (see Bacillus coli communis)
  - of Friedländer (see Bacillus pneumoniæ)
  - of glanders (see Bacillus mallei)
  - of influenza (see Bacillus influenzæ)
  - of leprosy (see Bacillus lepræ)
  - of malignant edema (see Bacillus œdematis maligni)
  - of ozena,, probably same as B. pneumoniæ, *q. v.*)
  - of pertussis, 337
  - of rhinoscleroma, 316
  - of Shiga (see Bacillus dysenteriæ)
  - of smegma, 33, 143
  - of soft chancre (see Bacillus of chancroid)
  - of syphilis, Lustgarten, 171
    - Joseph and Piorkowsky, 171
  - of tetanus (see Bacillus tetani)
  - of typhoid fever (see Bacillus typhi abdominalis)
  - of Vincent, 275, 349
  - of whooping cough, 337
  - of xerosis, 343
  - paracolon, 313
  - paratyphoid, 313
  - pestis bubonicæ, 320
    - cultural characters, morphology, pathogenic properties, 320
    - toxins from, 190
    - vaccine for, 202
  - phlegmones emphysematosæ, 326
  - phosphorescens Indicus, 271
  - pneumoniæ, Friedländer, cultural characters, morphology, pathogenic properties, 314
    - in suppuration, 284
  - prodigiosus chromogenic properties, 128
    - cultural characters, morphology, 269
    - with streptococcus pyogenes for treatment of sarcoma, 399
  - proteus, cultural characters, morphology, 219 319
    - in botulism, 159
    - in suppuration, 284
    - mirabitis vulgaris, Zenkeri, 319
- Bacillus pseudodiphtheriæ, 343
  - pyocyaneus, cultural characters, morphology, 317
    - in suppuration, 284
  - pyogenes fœtidus, detection of, in water, 145
    - dissemination by water, 139
    - identity with B. coli communis, 381
    - in suppuration, 284
  - ramosus, (see B. mycoides)
  - smegmæ preputialis, 33, 36, 163
  - subtilis, 271
  - tetani, cultural characters, morphology, 350
    - in soil, 135
    - isolation of, by heat, 94
  - tuberculosis, acclimatization of, on artificial media, 81, 352
    - avain, 362
    - in milk, 151
    - in suppuration, 285
    - location of, in animal tissues, 352
    - morphology, cultural characters, pathogenic properties, 350
    - of birds, 362
    - peculiar staining properties, 33, 350
  - typhi abdominalis (*sive typhosus*), addition of substances to water to facilitate detection of, 147
    - contrasted with colon, 382
    - cultural characters, morphology, 370
    - diagnosis of, by Widal test, 376, 193
      - by cultures, 371
    - infection of embryo with, 172
    - in ice, 148
    - in suppuration, 285
    - presence in milk probable, 152
    - reported presence in water, 145
  - vaginalis (Döderlein), 163
  - violaceus, 270
- Bacteria, acid-proof, 33, 36
  - acclimatization of, to artificial media, 81
  - adherence to moist surfaces, 80
  - aerobic, 127
  - anaerobic, 127

Bacteria, antitoxins for, 200
    diphtheria antitoxin, 346
    tetanus antitoxin, 331
  appearance of, on culture media, 7
  appearance of protoplasm of, 121
  appearance under the microscope of, 7
  avenues of entry of, into the body, 172
  behavior of, on various media, 81, 113
  capsules around, appearance of, 49
    apparent nature of, 121
  chlorophyll, relation to, 1
  chromogenic, 128
  classification of, 117
  cultivation of, 94
  coagulation of milk by, 129
  cover-glass preparations of, 27, 29
  decomposition of cellulose, 129
    of fat, 129
    of urine, 129
  definition of, 3
  diseases caused by, 170
  distribution, 4
  early observation of, 10
  early speculations about, 10
  effects of agitation on, 246
    of cold on, 246
    of desiccation on, 246
    of disinfectants on, 247
    of electricity on, 247
    of heat on, 125, 247
    of moisture on, 126
    of oxygen on, 127
    of preservatives on, 159
    of pressure on, 247
    of Röntgen rays on, 247
    of sunlight on, 247
  end-products of growth of, 129
  endotoxins of, 186
  enzymes of, 128
  ferments formed by, 128
  flagella on, 125
  fluorescent, 128
  forms of, 3
  grouping of, 4, 118
  harmless, 5, 120
  higher, compared with lower, 118
    certain special, 276
  in expired air, 162
  in feces, 165
  in fish, 159
  in stools of children, 165

Bacteria, inoculation of culture media with, 78
  inversion of starch by, 129
  inversion of cane-sugar by, 129
  involution forms, 121
  isolation of, by animal inoculation, 94
    by dilution, 94
    by Esmarch roll-tube, 99
    by heating spore-bearers, 94
    by plating in Petri dishes, 96
  liquefaction of gelatin by, 128
  method of studying, 21, 113
  modification of pathogenic power, 127
  moisture, in relation to growth of, 126
  morphology of, 3, 117
  motility of, 124
  multiplication of, 122
  nitrifying, 5, 126
  non-pathogenic, 267
  number of species of non-pathogenic, 267
  of air, 137
    alimentary canal, 164
    the conjunctiva, 161, 162
    the cranial sinuses, 160
    the external genitals, 163
    the Fallopian tubes, 160
    the gall-bladder, 160
    the ground-water, 139
    the intestines, 164
    the mouth, 161
    (B. buccalis maximus), 277
    (B. pneumoniæ Friedlander), 315
    (Leptothrix buccalis), 277
    (Leptothrix innominata), 277
    (Leptothrix maxima buccalis), 277
    (M. lanceolatus), 305
    (S. dentium), 274
    the nasal cavity, 161
    the normal human body, 160
    the urethra, 162
    the vagina, 162
  part played by, in nature, 5
  pathogenic, classification of, 282
    definition of, 120
  phosphorescent, 128
  physiology of, 117
  products of growth of, acids, 131
    enzymes, 128

Bacteria, products of growth of, gases, 131
  indol, 129
  pigment, 128
  ptomaines, 153, 159
  toxins, 186
 pure cultures of, 94
 putrefactive, 94
 relation of oxygen to, 127
 resemblance to other living forms, 1
 size, absolute, 4
  variability, 121
 spontaneous generation of, disproven, 3, 12
 spore formation, 14
 synonyms for, 4
 staining of, in cover-slips. 28
  in sections of tissues, 37, 41
  in sputum, 34, 350
 thermal death-point of, 125
 thermophylic, 125
 transmission of specimens by mail, 110
 useful, 5, 120
 warmth in relation to growth of, 125
 zoögloea masses of, 122
Bacterial poisons in cheese, meat, milk and other foods, 186
Bacteriolysis, by normal and of immune blood-serum, 193
 source, 218
 theories in regard to the composition of lysins in general, 226
 mechanism of, 230
Bacterium acidi lactici, 316
 aerogenes, 316
 coli communis (see Bacillus coli communis)
 pneumonicum, 316
 syncyanum (see Bacillus cyanogenus), termo, definition, 134 (see also Bacillus proteus)
 ureæ, 273
 Zopfii, 273
Bail on aggressins, 217
Balsam, Canada, for mounting cover-slip preparations, 27, 29
Basic anilin dyes, 28
Basophylic granules, 41, 43
Bed-bugs, diseases carried by, 177
Beggeatoa, 276
Beef-tea, 64
Beri-Beri, 170
Berkefeld filter, 63
Bichlorid of mercury as germicide, 248
Biedert method for examining sputum, 37
Birds, tuberculosis of, 362
Bismarck brown as contrast stain with Gram's method and other methods, 28, 30
Black death, 325
Black-leg vaccine, 202
Blood-agar for cultivating B. influenzæ, 336
 M. gonorrheæ, 312
Blood, cultures from, 108
Blood-poisoning, 286
Blood-serum, agglutinins in, 190
 aggressins in, 217
 effect of dilution upon, 233
 effect of heating on bacteriolytic, potency of, 195
 of standing, on potency of, 194
 bactericidal properties of, 193
 cytolytic properties of, 194
 heterologous and homologous, 197
 inactivated, 195, 222
 Löffler's, 75
 lysins in, 193
 opsonins in, 216
 precipitins in, 196
 preparation of, for culture medium, 73
 reactivated, 195, 222
 specific precipitins for, 196
 sterilization of, for culture medium, 59, 74
 test for typhoid fever, 193
Blue milk, bacillus of (see Bacillus cyanogenus),
 pus, 318
 vitriol, 258
Bodily conditions predisposing to infection, 178
Boiling, sterilization by, effect on spores, 247
 efficacy of, 55
 for sterilizing water, 142
 for surgical instruments, 266
Boils, caused by S. pyogenes aureus, 293
Boöphilus annulatus, 177
Bordet's theory of nature of lysins, 226
 of toxin-antitoxin reaction, 225
 on difference between his and Ehrlich's theories, 229

Bordet's theory on bactericidal action of ox-serum, 236
  on dilutions of serum, 233
Boric acid (see Acid, boric)
Bouillon, 64
  sugar-free, 67
Bovine tuberculosis, danger of, in milch cows, 151
  lesions of, 355
Branching forms of bacilli, 119
Bread-paste, 76
Bromin as a germicide, 256
Bronchitis, B. influenzæ in, 337
  streptococci and pneumococci in, 289
Brownian movement, 24
Bubonic plague, bacillus (see Bacillus pestis bubonicæ)
Buchner's method for cultivating anaerobes, 90
Burner, Koch's safety, 86
Butter, tubercle bacilli in, 151
Butyric acid (see Acid, butyric)

Cadaver, care of, in contagious diseases, 259
Calcium compounds as germicides, 256
Canada balsam for mounting cover-glass preparations, 27, 29
  for mounting sections, 41
Capaldi's culture-medium, 347
Capsule bacillus of Pfeiffer, 315
Capsules of bacteria, definition of, 121
  nature of, staining of, by Hiss' method, by Welch's method, 49
Carbol-fuchsin for staining tubercle bacilli, 34
Carbolic acid (see Acid, carbolic)
Carbon dioxid, as a product of bacterial growth, 132
Carbuncles caused by S. pyogenes aureus, 293
Carmine, as contrast-stain in Gram-Weigert's method, 43
  in gentian-violet preparations, 45
Caries of the teeth, 162
Carriers of infection, 175
Caseation, 357
Catgut, surgical preparation, difficulty of sterilizing, 265
Cedar-wood oil, for immersion-lens, 20
Celloidin imbedding, 28
Cells, epithelioid, giant, 357
  pus, 283
Cellulitis caused by bacillus aerogenes capsulatus, 326
  by streptococcus pyogenes, 296
Cellulose, decomposition by bacteria in the stomachs of ruminants, 129, 165
Centrifuge for milk separator, 154
Cerebro-spinal meningitis, 309
Chancroid, bacillus of, 314
Charbon (see Anthrax)
  symptomatique (see Symptomatic anthrax)
Cheese-poisoning, 153
  chemical nature of antitoxic action, 220
Chemotaxis, definition, 125
  in inflammation, 214
  in suppuration, 282
Chicken-pox, 170
Chlorid of lime, as germicide, 256
Chlorin as a germicide, 255
Chloroform as a preservative for blood-serum, 74
Chlorophyll, absence of, in bacteria affects their nutrition, 126
  not present in bacteria, 1, 167
  in higher plants, 134
Cholera, Asiatic, diagnosis, 396
  B. dysenteriæ in, 387
  B. proteus, 320
  infantum, 320
  nostras, 401
  red reaction, 392
  spirillum (see Spirillum of Asiatic cholera), 390
Chromogenic bacteria, 128
Cladothrix, 276
Classes in bacteriology, hints for teaching, 113
Classification of bacteria, 117
Cleaning fluid, 25
Climate, influence on infections, 179
Clostridium butyricum, definition, 124
Coal-oil for destroying insects, 258
Cold, effect of, on growth of bacteria, 124
Collection of material for bacteriological examination of sputum, of urine, 107
  in suspected diphtheria, 109
Collection of samples of water for bacteriological examination, 142
Collodion, capsules, for cultivating bacteria inside the animal body, 106

Collodion, for imbedding tissues, 38
Colon bacillus (see Bacillus coli communis)
Colonies of bacteria, appearance of, examination of, how obtained, 100
Comma bacillus of cholera (see Spirillum of cholera)
  shaped bacteria, 118, 120
Complement analogy with enzymes, 229
  diversion of, 233
  effects of heat on, 195
  mode of action of, 228
  where found, 232
Condenser, Abbé, 20
Conjunctivitis, gonorrheal, 314
Consumption, 355
Contagious disease, definition, 169
  diseases, disinfection after, 260
Contrast stain, acid fuchsin, 28
  Bismarck brown, 28, 31, 37
  eosin, 28, 31, 48
  for tubercle bacilli, 33
  hematoxylin, 44
  picric acid, 28
  methylene-blue for carbol-fuchsin preparations, 37
Copper sulphate, 258
Copperas, 258
Cornet forceps, 25
Corrosive sublimate, 248
Cotton, absorbent, for anaerobic cultures, 91
  as plugs for test-tubes, 76
  power of filtering bacteria out of air, 13
Cover-glass forceps, 26
Cover-glass preparations, fixing of, 27
  impression preparations, 25
  smear preparations, 26
  staining of, 28
Cover-glasses, cleaning fluid for, 25
  handling of, 25
Cowpox, discovery of protective value of, for small-pox, 11
  used for vaccination, 200
Cranial sinuses, bacteria of, 160
Cream ripening, 151
Creolin, 251
Cresol, 251
Croup, membranous, 345
Croupous pneumonia, diagnosis of, 301
Croupous pneumonia, due to pus cocci, 288
  B. pneumoniæ, 315
  M. lanceolatus, 305
Cultivation of amebæ, 410
  of bacteria in collodion sacs, 106
  in test-tubes, 78, 101
  on plates, 95
  under anaerobic conditions: by Buchner's method, 91
    by Esmarch's method, 92
    by Frankel's method, 91
    by Hüppe's method, 93
    by Koch's method, 93
    by Liborius's method, 92
    by Novy's method, 92
    by Park's method, 93
    by Wright's method, 91
    of trypanosomes, 409
Culture media, agar-agar, plain, 69
    with blood-serum, 75
    with dextrose, with lactose, with saccharose, 71
    with glycerine, 70
  blood-serum, 73
  bouillon, plain, 64
    with dextrose, with lactose, with saccharose, 67
  sugar-free, 64
  Löffler's mixture, 75
  milk, 72
  nitrate broth, 73
  object of sterilizing, 64
  preparation of test-tubes for, 76
  sterilization of blood-serum, 74
    of ordinary media, 55, 58
    of milk, 72
    of sugar media, 67
    of test-tubes for culture media, 76
Cultures at autopsies, upon animals, 104
  on human cadavers, 109
  destruction of, 116
  from the blood, 108
Cupric sulphate, 258
Cutting sections of tissues, 40
Cystitis, caused by B. coli, 290
  by B. lactis aerogenes, 386
  B. proteus in urine in, 320
Cytolysins, 193
Cytolysis, 193
Cytolytic potency of blood-serum, 194

Decomposition, 129
Delafield's hematoxylin, 44
Deneke's spirillum, 402
Dengue, 170, 172
Dental caries, 162
Deodorizers, 238
Dermatitis, blastomycetic, 281
Dextrose-agar, 71
Dextrose-bouillon, 67
Diagnosis of actinomycosis, 368
  of bubonic plague, 330
  of cholera, 396
  of diphtheria, 297, 338
  of dysentery (from cultures), 387
    (serum test), 388
  of glanders (Straus' method), 365
  of gonorrhea, 312
  of influenza, 337
  of malaria, 432
  of Malta fever, 308
  of meningitis, cerebro-spinal, 310
  of pneumonia, 306
  of tuberculosis (tuberculin injection), 261
    (microscopic examination), 251
  of typhoid fever, (appearance of culture), 371
    (Gruber-Widal test), 376
    (from stools), 378
Diphtheria, antitoxin (behavior of toxin-antitoxin mixtures), 223
    methods of production), 209, 346
  bacillus of (accidental infection, 115
    (cultural characters and morphology), 338
    (in throats of healthy persons), 175
  diagnosis, 338
  toxin (nature), 187
    (analogy with ferments), 189
    (effect on animals on injection), 209
    (mixed with antitoxin), 223
    (death due to), 345
Diphtheritic membrane, location, 345
Diplococcus, definition, 119
  intercellularis meningitidis (cultural characters and morphology), 309
    (in suppuration), 284
  of gonorrhea, 311
  of pneumonia (see Micrococcus lanceolatus)
Disease, bacteria in, 170
Diseases caused by bacteria, 170
  by protozoa, 425
  probably due to microörganisms, 171
  infectious, immunity from, 199
  dissemination of: by fleas, flies, bed-bugs, 176
    by mosquitoes, ticks, 177
Disinfectants, definition, 239
  effects of the character of the medium in which the tests are made upon, 241
  elective affinity of certain, 241
  methods of testing the potency of, 242
  specific action of certain, 241
  testing of the potency of gaseous, 245
  determination of the potency of, 242
  physical, 246
  chemical, 247
Disinfection at autopsies, 259
  definition of, 238
  of cadavers after infectious disease, 260
  of dejecta, 258
  of hands, 264
  of houses, 260
  of mouth, 258
  of sputum, 259
  of stools, 259
  of urine, 259
  surgical, 252
Distribution of bacteria in nature, 135
Diversion of complement, 233
Dorset's egg-medium, 76
Dressings, surgical preparation, 265
Drigalsky-Conradi's method for detecting typhoid bacilli in water, 374
Drinking water, purification of, 140
Drying, influence on bacteria, (see Bacteria, effect of desiccation upon)
Ducrey's bacillus, 314
Dunham's peptone solution, 73
Dyes, aniline, 28
  as germicides, 251
  for bacterial stain, 28
Dysentery, ameba of, 415
  B. dysenteriæ in, 386
  B. pyocyaneus in, 318

Ear, middle, 160, 289
  inflammation of, 289
  normally sterile, 160
Eberth's bacillus (see Bacillus of typhoid fever)
Edema, malignant, bacillus (see Bacillus œdematis maligni)
Egg-albumen as a culture-medium, 75
Egg-medium of Dorset, 76
Eggs, in cultivating anaerobes, 75, 82
Ehrlich's side-chain theory, 218
Electric heater and regulator for incubators (Rogers), 87
Electricity, influence on bacteria, 247
Elsner's culture-medium, 374
Embryo, infection of, 172
Emmerich's bacillus (see Bacillus coli communis)
Emphysematous gangrene (see Bacillus aerogenes capsulatus), 325
Endocarditis, M. gonorrhϾ in, 311
  M. lanceolatus in, 306
  pyogenic bacteria in, 288
  S. pyogenes aureus in, 293
  streptococcus pyogenes in, 296
Endogenous spores, 122
Endotoxins, 186
Enzymes, analogy with toxins, 189
  bacterial, 128
Eosin in Wright's stain, 48
Epithelioid cells, 357
Epitoxoid, 224
Epitoxonoid, 224
Erysipelas (see Streptococcus of erysipelas)
Escherich's bacillus (see Bacillus coli communis)
Esmarch's method for anaerobes, 92
  roll-tubes, 99
Essential oils as germicides, 257
Eye-piece, 18, 19, 20

Fallopian tube, bacteria of, 160
Farcy-buds, 365
Fats, decomposition by bacteria, 129
Favorable and unfavorable conditions for growth of bacteria, 125
Feces, bacillus of tetanus in, 329
  bacteria of, 165
  disinfection of, 259
  typhoid bacilli, examination for, by serum test, 376
    in water or feces, 373
Fermentation, 12, 133
  tube, 132
Ferments, bacterial, 128
  and toxins, 189
Ferrous sulphate, 258
Fibrin, Weigert's stain for, 42, 258
Ficker-Hoffmann's method for detecting typhoid bacilli, 374
Film-preparations, making of, 26
  fixing of, 27
  staining of, 28
Filter, alum, 141
  for bacteria, 62
  American, 141
  Berkefeld, 62
  infusorial earth, 62
  Kitasato, 62
  mechanical, 141
  Pasteur-Chamberland, 62, 142
  sand, 141
  unglazed porcelain, 142
Filterable microörganisms, 171
Filtration, sterilization by, 141
  of water, 63, 140
Finkler and Prior spirillum, 401
Fishing from colonies, 91
Fission, multiplication of bacteria by, 3
Fixation of cover-glass preparations, 26, 27, 28
  of slide-preparations, 27
  of tissues, 37
Fixative, glycerin albumen, 39
Flagella, as organs of locomotion, 125
  staining of, 50
Fleas, diseases carried by, 177
Flies, cholera carried by, 176, 395
  tuberculosis carried by, 176
  typhoid fever carried by, 176, 389
Fluid for cleaning, 25
Fluorescence of bacteria, in water, 128
  B. fluorescens liquefaciens, 269
  B. pyocyaneus, 317
Focusing the microscope, 21
  for hanging-drop preparations, 23
Fomites, definition, 169
Food used by bacteria, 126
Foods, bacteria of, 149
  poisoning by, 159
Foot-and-mouth disease, 171
Forceps, Cornet, 26
  cover-glass, 26
  for slides, Kirkbride, 27
  Stewart, 26
Formaldehyde as a germicide, 251
  candles, 254
  for disinfection of rooms, 260
  fixation of tissues with, 37

Formalin, 251
  lamps, 253
Formic acid (see Acid, formic)
Fowl-cholera, protective inoculation, 201
Fowls, tuberculosis of, 362
Fractional sterilization, 55
Fränkel's method for anaerobes, 92
  pneumococcus (see Micrococcus lanceolatus)
Freeman's pail for pasteurizing, 60
Freezing, influence on bacteria, 246
Friedländer's bacillus of pneumonia (see Bacillus pneumoniæ)
Fuchsin, as bacterial stain in general, 28
  acid as contrast-stain, 28
  carbol for tubercle bacilli, 34
Fürbringer's method for disinfecting hands, 264
Fusiform bacillus of Vincent, 275

Gabbett's method for staining tubercle bacilli, 35
Gall-bladder, colon bacillus in, 384
  typhoid bacilli in, 378
  usually sterile, 160
Gangrene, emphysematous (see Bacillus aerogenes capsulatus, 325
Gas-burner, Koch's, 86
Gas-formation by bacteria, 131
  phlegmons, 327
  regulator, mercurial, Reichert, Roux, 84
Gastric juice, germicidal power, 164
Gelatin, nutrient, 68
  liquefaction, 128
  tetanus bacilli in, 329
Gélose (see Agar-agar)
Gentian-violet, 31
Geppert's test for germicides, 244
Germicidal power of blood-serum, due to lysin, 166
  effect of dilution upon, 233
  inactivated by heat, 222
  nature of, 226
  reactivation of, 222
Germicide, definition, 239
  determination of the potency of, 242
  effects of the character of the medium in which the tests are made upon, 241
  elective affinity of certain, 241
Germicide, methods of testing the potency of, 242
  specific action of certain, 241
  testing of the potency of gaseous, 245
Germicides, tests for potency of, 242
Germ, use of the word, 3
German measles, 170
Giant-cell, 357
Glanders bacillus, morphology, cultural characters, 364
  Straus's method for diagnosing, 365
Glassware, sterilization of, 53
Gloves, rubber, 265
Glucose, agar, 71
  bouillon, 67
Glycerin-agar, 70
  albumen, 40
  bouillon, 67
Gonococcus of Neisser, in suppuration, 284
  morphology, cultural characters, 311
Gram-Günther method, 32
Gram's method for cover-glass preparations, 31
  for sections, 42
  bacteria stained by, 32
    not stained by, 32
Gram-Weigert method, 42
Gray tubercle, 358
Green pus (see Bacillus pyocyaneus)
Ground-water, 139
Group agglutinins, 191
  lysins, 194
  precipitins, 197
Groups of bacteria, 118
Gruber-Widal reaction, 193
Guarnieri's medium, 75
Gun-cotton, 38

Haffkine's inoculations for plague, active immunity produced by, 212
  cultures employed in, 323
  preparation of vaccine, 202
Hair-follicles, infection around, 286
Hands, disinfection, 264
Hanging-block, 24
  drop, 23
Haptophore, 219
Hardening of tissues, in alcohol, in formalin, 37
Hay bacillus, for class work, 114

Hay morphology, cultural characters, 271
  resisting power of spore to heat, 123
Heat, effect on growth of bacteria, 247
  sterilization by, 53
Hematoxylin, 44
Hematozoön of malaria, 432
Hemolysis, 194
Healthy persons as carriers of infection, 175
Heterologous serum, 191
High temperature incubator, 82
Higher bacteria, 276
Hill's test for germicides, 243, §2
Hiss, medium of, 373
  stain for capsules, 49
Historical sketch of bacteriology, 8
Hog cholera, 171
Holmes, O. W., 12
Homologous serum, 191
Honing of knives, 40
Hot-air sterilizer, 54
Houses, disinfection, 253, 260
Hueppe's method for anaerobes, 82
Hydrochloric acid (see Acid, hydrochloric)
Hydrogen, cultivation of anaerobes under, 80
  peroxide, 257
  sulphide, 119
Hydrophobia, organism, 170
  diagnosis of, 203, 208
  effect of radium rays on virus of, 206
  incubation period in, 207
  preventive inoculation, 206
  "virus fixe," 206
Hypersensibility to infection, 11
Hypha, 280
Hypochlorite of calcium, 256
Hypodermic inoculation of animals, 102

Ice, bacteria of, 148
Ice-cream poisoning, 153
Illumination for the microscope, 21
Imbedding, in celloidin, in paraffin, 38
Immune-body, 226
Immunity, acquired, 200
  active, 211
  antitoxic, 208
  bacteriolytic, 193
  by injection of cultures, 201
  duration of, 212
Immunity, individual, 198
  following vaccination, 200
  scope of, 198
  natural 199
  passive, 211
  racial, 199
  side-chain theory, 218
  theories of, 212
  unit, 348
Impression-preparation, 25
  Inactivated serum, 222
Incubator, 82
Indol, formation of, by bacteria, 129
  test for, 129
Infected surgical wounds, 262
Infection, auto-, 177
  avenues of, 172
  bodily conditions favoring, 178
  by air, 175
  by bed bugs, 177
  by flies, 176, 380
  by healthy individuals, 175
  by insects, 177
  by mosquitoes, 172, 177
  in milk, water, soil, 176
  influence of amount of material on, 182
    of virulence on, 182
  local conditions favoring, 181
  mixed, 183
  modes of, 172
  of embryo, 172
  of investigators with pathogenic bacteria, 115
  of wounds, 263
  predisposing causes: Age, altitude, climate, individual, 179
  racial, 180
  secondary, 183, 287
  terminal, 183
Infectious disease, definition, 168
  diseases not followed by immunity, 200
Inflammation, 283, 288
  diphtheritic (see Pseudo-membranous inflammation)
Influenza bacillus, 336
Infusorial earth in filters, 62
Inoculation of animals, for testing pathogenic property of bacteria, 103
    for isolation of bacteria, 94
    intravenous, 103
    subcutaneous, 102
  of tube-cultures, 79

Inoculations, preventive, 201
  for anthrax, 201
  for black-leg of cattle, 16, 202
  for bubonic plague, 323
  for cholera, 394
  for erysipelas of swine, 202
  for fowl-cholera, 201
  for hydrophobia, 203
  for small-pox, 10, 200
  for tuberculosis, 362
  for typhoid fever, 381
Insects, destruction of, 255, 258
  infection spread by (see Infection)
Instruments, surgical preparation, 266
Intermittent sterilization, 55
Intestine, bacteria of, 164
Intravenous inoculation of rabbits, 103
Invisible microbes, 121
Involution forms of bacteria, 121
Iodide of mercury, 249
Iodine solution, in Gram's stain, 31
  in Weigert's stain, 41
Iodoform, 257
Iris diaphragm, 18
Isolation of bacteria: by animal inoculation, by dilution, by heating, by plating, 94
  by Esmarch roll-tubes, 99
  historical, 15
Itch, 15

Jenner, 11
Journals of bacteriology, 8

Kerosene, for destroying insects, 258
Kirkbride forceps for slides, 27
Kitasato filter, 62
Klatschpreparat (impression preparation), 25
Klebs-Löffler bacillus (see Bacillus diphtheriæ)
Knives, sharpening of microtome, 40
Koch, discovery of B. tuberculosis by, 16
Koch's gas-burner, 86
  method for anaerobes, 93
    plate-cultures, 15
      as means of isolating bacteria, 94
      (historical), 15
      use of, at autopsies, 105
  postulates for proving the pathogenic property of bacteria, 168
Koch's steam sterilizer, 59
  tests for germicides, 243

Lactic acid (see Acid, lactic)
Lactose, agar, 71
  bouillon, 67
Leeuwenhoek, 9
Leprosy bacillus, 362
  morphology, cultural characters, pathogenic properties, 362
  resemblance to B. tuberculosis, 351
Leptothrix (in normal mouth), 161
  (morphology, cultivation of), 277
  buccalis, innominata, maxima buccalis, 277
Leucin, as product of bacterial growth, 129
Leucocytosis, artificial, 215
  in disease, 214
Leucomaines, 188
Ligatures, surgical preparation, 265
Light, influence on bacteria, 247
Lime as a germicide, 256
Liquefaction of gelatin, 128
  analogy to trypsin digestion, 128
  as a means of classification, 129
Lister, 14
Lithium-carmine, 45
Litmus-agar, 71
  milk, 73
Lockjaw (see Tetanus)
Löffler's bacillus of diphtheria (accidental infection from cultures), 115
    (in throats of healthy persons), 175
    (morphology, cultural characters, pathogenic properties), 338
    (see also Bacillus diphtheriæ)
  blood-serum, 75
  methylene-blue, 30
  stain for flagella, 50
Lump-jaw, 366
Lungs, bacteria of the, 160
Lustgarten's bacillus of syphilis, 171
Lymphoid tissues, relation of bacteria to, 160, 173
Lysins, nature of ,193
  inactivated, 195
  reactivated, 195
Lysol, 251

Macrophages, 212
Madura disease, Madura foot, 369
Magnifying power of objectives, 21
Mails, transmission of specimens of bacteria in, 110
Malachite-green as a germicide, 251
Malaria, destruction of mosquitoes of,
    with sulphur dioxid, 255
    with petroleum, 258
  mosquitoes as carries of, 177
  parasite of, 432
Malarial parasite, preparation of blood-films, 108
  staining of, in blood-films, 46
Malignant edema, bacillus in soil, 135
  morphology, cultural characters, pathogenic properties, 327
  pustule (see Bacillus anthracis)
Mallein, extract from cultures, 190
  method of preparation, 366
Malta-fever, micrococcus of, 307
Marmorek's antistreptococcus serum, 298
Massachusetts State Board of Health steam sterilizer, 58
Mastzellen, 41
Mayer's glycerin-albumen, 40
Measles, complicated with diphtheria, 246
  microörganism undiscovered, 170
  streptococcus in, 298
Meat, tubercle bacilli in, 152
Mechanical filtration of water, 141
Medium, culture (see Culture-medium)
Membranous croup, rhinitis, 345
Meningitis, streptococcus pyogenes in, 297
  M. lanceolatus in, 306
  diplococcus intracellularis meningitis in, 309
  B. pneumoniæ in, 315
Mercuric chloride (see Bichloride of mercury).
  iodide, 249
Mercurol, 250
Mercury bichloride of mercury (see Bichloride)
Metachromatic granules of bacteria, 121
Metastatic abscesses, 290
Metchnikoff, theory of phagocytosis, 212
  vibrio of, 400
Methyl-alcohol lamp in formaldehyde disinfection, 254
Methylene-blue, as a stain for bacteria, 28
  as a germicide, 251
  Löffler's, 30
Methyl-violet as a germicide, 251
Miasmatic disease, definiton, 169
Microbe, use of the word, 3
Micrococcus agilis, 268
  amylovorus, 168
  definition, 118
  gonorrhœæ, in auto-infection, 178
    morphology, cultural characters, pathogenesis, 311
  lanceolatus changes on artificial cultivation, 302
    diseases caused by, 306
    habitat, 305
    in normal human mouth, 161
    in suppuration, 284
    means of differentiation from streptococcus, 392
    method of staining capsules of, 131
    morphology, cultural characters, pathogenesis, 301
  melitensis, 307
  of sputum septicemia (see Micrococcus lanceolatus)
  pneumoniæ crouposæ, 249 (see Micrococcus lanceolatus)
  pyogenes tenuis (see Strepococcus pyogenes)
  tetragenus, in suppuration, 284
  morphology, cultural characters, pathogenesis, 300
  ureæ, 268
Micromillimeter, 21
Micron, μ, 21
Microphages, 212
Microscope, 18
Microscopic examination of bacteria, 21
Microtome, 39
Miliary tubercle, 358
  tuberculosis, 358
  effect of centrifugalizing, 154
    of temperature on bacterial growth in, 152
Milk as a culture-medium, 72
  bacteria of, 149
  fermentation in, 152
  germicidal properties of, 155
  human pus cocci in, 152
  hygienic production of, 158
  lactic acid in, 152

Milk, methods of determining number of bacteria in, 149
  number of bacteria in, 149
  of lime, 256
  pasteurization, effect of, on pathogenic bacteria in, 154
    definition of, 60
  pathogenic bacteria in, 151
  poisoning, 153
  pus cocci in, 152
  recommendations of American Public Health Association for the examination of, 149
  samples of, 97
  scarlet fever conveyed by, 152
  sources of contamination of, 151
  sterilization in infant feeding, 153
  tubercle bacilli in, 151, 153
Miller's spirillum, 326
Milzbrand (see Anthrax)
Mixed infection, 183
Mixtures of toxin and antitoxin, 223
Modes of enrty into the body, 172
Moisture in relation to growth of bacteria, 126
Morphology of bacteria, 177
Mosquitoes as carriers of infectious disease: malaria, 177, 434
  yellow fever, 440
  destruction of (with coal oil), 258
    (with sulphur dioxide), 255
Motility of bacteria, 124
Moulds, aspergillus, mucor, oidium, penicillium, 278
  cultivation, 76
  description of, 278
  for class work, 115
  in air, 137
Mouth, B. Friedländer in, 315
  characteristic bacteria in normal, 161
  leptothrix in, 277
  M. lanceolatus in, 301, 305
  spirilla in, 274
Movement, Brownian, 24
Mucor mucedo, 279
Mucous membranes, bacteria of, 160, 161
Multiplication of bacteria, 122
Mumps, microörganism of, not known, 170
Mustard as a deodorizer, 257
Mycelium, 280
Mycetoma, 369
Nasal cavity, bacteria of, normally, 161
  B. rhino scleroma, 316
Natural immunity, 199
Necrosis caused by toxin, 189
Neisser's gonococcus (see Micrococcus gonorrhœæ
  stain for diphtheria bacilli, 338
Neisser-Wechsberg phenomenon, 233
Neutral red in culture-media, 71
  in diagnosis of B.coli, 372, 386, 387
Neutralization of culture-media, 65, 66
Nitrate of silver as a germicide, 250
  as a stain for S. obermeieri, 405
Nitrifying bacteria in soil, 131
  nutrition of, 126
Nitrites, conversion of, into nitric acid, 131
Nitrogen fixation by bacteria, 136
  liberation by bacteria, 132
Nitroso-indol reaction, 132
Noma, 277
Non-pathogenic bacteria, classification of, 267
Normal solutions, 66
Nose-piece to microscope, 18
Novy's method for anaerobes, 32
Number of bacteria in feces, 165
    milk, 151
    soil, 135
    water, 139
  species of non-pathogenic bacteria, 267
Nutrient agar-agar, 69
  bouillon, 64
  gelatin, 68
Nutrition of bacteria, 126

Obermeier's spirillum, 404
Objectives, 18
Ocular, 18
Odors developed by bacteria, 132
  from water, 139
Oese, 22
Oidium lactis, 279
Oil, aniline, for Gram's method, 30
    for Weigert's method, 42
  cedar-wood, 20
  immersion objective, 19
  kerosene, 258
Oils, essential, as germicides, 257
Opsonin, 216
Osteomyelitis, S. pyogenes in, 293
  B. typhosus in, 379
Otomycosis, 281

Ovum, bacteria conveyed in, 172
Oxalic acid (see Acid, oxalic)
Oxygen, relation of bacteria to, 127
Ox serum in relation to cytolysis, 235
Oysters, typhoid fever conveyed by, 158
Ozena bacillus (probably identical with B. pneumoniæ), 315
Ozone in purifying water, 142

Paracolon bacillus, 384
Paraffin imbedding, 38
Paraform or paraformaldehyde, 252
Paraplague bacillus, 325
Parasite, definition, 120
Paratyphoid bacillus, 384
Parietti's method for examination of water, 147
Park, W. H., method for cultivating anaerobes, 93
Passive immunity, 211
Pasteur, hydrophobia virus, 206
  inoculation of attenuated cultures, 16
  microörganisms, the cause of fermentation, 13
Pasteur-Chamberland filter, 63
Pasteurization, definition, 60
  effect on B. tuberculosis in milk, 153
Pathogenic bacteria, definition, 282
Pear-blight, 6, 168
Penicillium glaucum, 278
Peptone, as product of bacterial growth, 129
  Dunham's solution, 73
  enriching fluid, 399
  in nutrient bouillon, 64
Peptonizing ferments formed by bacteria, 129
Pericarditis, micrococcus lanceolatus in, 306
  staphylococcus pyogenes aureus in, 288, 294
  streptococcus pyogenes in, 296
Periositis, due to B. typhi, 379
Peritonitis, due to B. coli, 288
  staphylococcus pyogenes aureus, 294
  streptococcus pyogenes, 296
  B. pyocyaneus, 318
  B. proteus, 319
  colon group, 384
Perlsucht, 289
Permanganate of potassium, 206, 211
Peroxide of hydrogen, 205
Petri dishes, 96
Petroleum for destroying insects, 258
Pfeiffer's capsule bacillus, 315
Pfeiffer's phenomenon) 228, 394
Phagocytosis, as means of defense against infection, 282
  definition, 212
  melaniferous, 437
Phenol (see Acid, carbolic)
Phenolphthalein in titration, 66
Phosphorescence of bacteria, 128, 271
Physiology of bacteria, 117
Picric acid (see Acid, picric)
Piorkowski's culture-medium, 374
Piroplasma, 177, 440
Placenta, bacteria transmitted through, 172
Plague, bubonic, bacillus of (see Bacillus pestis bubonicæ)
Plants, diseases of, 6, 168
Plasmodium of malaria, morphology, cycle of development, 432
  preparation of blood-films, 108
  staining of, 45
Plasmolysis, 121
Plate-cultures, 95
Platinum wire, rules for use, 22, 78
Pleuritis, streptococcus pyogenes aureus in, 288, 294
  streptococcus pyogenes in, 296
  micrococcus lanceolatus in, 306
Pleuro-pneumonia of cattle, 150
  invisible microbes, 171
Plugs, cotton, for tubes, 76
  for anaerobic cultures, 91
Pneumococcus of Fränkel (see Micrococcus lanceolatus)
Pneumonia, broncho-, due to acid-proof bacteria, 370
  to B. pneumoniæ, 315
  to B. pycyaneus, 318
  to B. typhosus, 379
  to pus cocci, 288
  croupous (see Croupus pneumoniæ)
Pneumonomycosis, 281
Poisoning by food, milk, ice cream, cheese, 153
  oysters, fish, meat, 159
Porcelain filter, 62
Post-mortems, disinfection at, on animals, 95
  on human beings, 259

Post-office rules for mailing specimens of bacteria, 110
Potassium permanganate, 257
Potato as a culture-medium, 71
  growth of B. typhosus on, 372
  bacillus (see Bacillus mesentericus vulgatus)
Precipitins for albumen, 196
  for bacteria, 197
Predisposition to infection, 155
  age, altitude, climate, individual, 179
  amount of infectious material, number of bacteria, virulence of bacteria, 182
  bodily conditions, general, 178, 181
  local, 285
  of different organs and tissues, racial, 180
Pressure, effect of, on bacteria, 247
Prevention and cure of bacterial diseases, 16
Products, bacterial: ammonium carbonate, fatty acids, indol, leucin, phenol, skatol, sugar, toxins, tyrosin, 129
  pigment, 128
  ptomaines, 159, 161
  tyrotoxicon, 153
Propionic acid (see Acid, proprionic)
Protargol, 250
Protective inoculation for anthrax, 201, 335
  Asiatic cholera, 394
  black-leg of cattle, 202
  bubonic plague, 202, 323
  erysipelas of swine, 202
  fowl-cholera, 202
  hydrophobia or rabies, 206
  rinderpest, 201
  small-pox, 10, 200
  tuberculosis, 362
  typhoid fever, 381
Proteus bacillus (see Bacillus proteus)
Protoplasm, appearance of bacterial, 121
Protoxoid, 224
Protozoa, pathogenic, in dysentery, malaria, 17
  Texas fever, 177
  trypanosomes, 408
  amebæ, 410
Pseudo-diphtheria bacillus, 343
Pseudo-gonococcus, 311
Pseudo-membranous inflammations due to B. diphtheriæ, 342, 345
  B. dysenteriæ, 307
  M. lanceolatus, 307
  streptococcus pyogenes, 296
Pseudo-pneumococcus, 307
Pseudo-tuberculosis, 362
Ptomaine poisoning, 159
Ptomaines, 186, 187
Puerperal fever, 11
  B. diphtheria in, 345
  S. pyogenes in, 296
Pure cultures, advantages of, shown by Koch, 16
  methods of obtaining, by animal inoculation by dilution, by heating, 94
  by plating, 104
Purification of water by chemicals, by mechanical filtration, 141
  by sand filtration, 140
Pus, blue, 318
  cells, 282
  collection of specimens for examination, 107, 109
  formation, 283
  green, 318
Putrefaction, 13, 133
Pyemia, 291
Pyocyanase, 190
Pyocyanin, 317
Pyogenic bacteria, as causes of various diseases, 288
  as secondary invaders, 287
  in inflammation of middle-ear, 289
  in pneumonia, 289
  in suppuration, 284
Pyoktanin, 250
Pyosalpinx, 314
Pyrogallic acid for cultivating anaerobes, 91
Pyroxylin, 38

Quarantine, early used by Italians, 9

Rabies, caustive agent unknown, 170
  diagnosis of, 208
  Pasteur treatment for, virus fixe, 206
Racial immunity, 199
  predisposition to infection, 179
Rats, acid-proof bacilli of, 362
  relation to bubonic plague, 322
Rauschbrand, vaccine for, 202

Ray-fungus of actinomycosis, in pus formation, 284
  morphology, cultural character and pathogenesis, 366
Reactions of culture-media, correction of, 68
  methods of determining, 65
  optimum, 67, 127
Reactivated blood-serum, 195, 222
Receptor, antitoxin, definition, 218
  first order, 221
  second order, 221
  third order, 223
Recovery from infectious disease, agents concerned in, 184
  influence on immunity, 199, 212
Regulators, gas, Reichart's, 84
  Roux, 85
  electric, Roger's, 87
Relapsing fever, spirillum (see Spirillum obermeieri)
Rennet-like bacterial ferments, 129
Rheumatism, microörganism doubtful, 170
  M. rheumaticus, pyogenic cocci in, 290
Rhinoscleroma, bacillus, 316
Ricin, analogy with bacterial toxins, 189
Ripening of cream, 151
Robin, analogy with bacterial toxins, 189
  production of antitoxin with, 209
Roll-tubes of Esmarch, 99
Rooms, disinfection with formalin, 253
  with sulphur dioxid, 254
Röntgen-rays, influence of, on bacteria, 247
Root-tubercle organisms, on leguminous plants, organisms of, 136
Rosolic acid, (see Acid, rosolic)
Rouget, 202
Rubber caps for culture-tubes, 86
  for use at autopsies, 110
  at surgical operations, 265
  gloves, 212
  stoppers for culture-tubes, 86

Sabouraud's culture-medium for fungi, 76
Saccharomyces cerevisiæ, 278
Saccharose, agar, 71
  bouillon, 67
Salt-agar, 264
Sanarelli's bacillus of yellow fever (see Bacillus icteroides)
Sand filtration of water, 140
Sapremia, 184
Saprophyte, definition, 167
Sarcina, definition of, 119
  pulmonum, 268
  ventriculi, 268
  in healthy stomachs, 164
  morphology, cultural characters, 268
Sarcoma, toxins of streptococcus for, 299
  complicated with diphtheriæ, 345
  microörganism of, not discovered, 170
  streptococcus in, 297
Scarlet fever, 170
Schizomycetes, definition, 4
Schultz's method for neutralizing culture-media, 65
Schweinerothlauf, vaccine for, 202
Scrofula, 358
Sealing culture-tubes for anaerobic cultures, 91
  to prevent drying out of culture medium, 86
Secondary infection, 183
  definition of, 183
  due to S. pyogenes, 287
Section-cutting, 40
Sections, stained with carmine, 45
  staining bacteria in, 41
    Gram's method, 42
    hematoxylin, 44
    tubercle bacilli, 43
    Weigert method, 42
Sedgwick's test for germicides, 244
Sedgwick-Tucker aerobioscope, 138
Self-purification of water, 139
Semen, transmission bacteria by, 172
Semmelweis, demonstration of infectious nature of puerperal fever, 11
Separator for milk, influence on number of bacteria, 154
Septicemia, 184
Serum (see Blood-serum)
  test for typhoid fever, value of, 193
  method employed in, 376
Shiga's bacillus of dysentery (see Bacillus dysenteriæ)
Side-chain theory of immunity, 218
Silk threads in testing germicides, 243

Silver nitrate, 250
    wire in surgery, 266
Size of bacteria, 4, 121
Skatol, produced by bacteria, 129
Skin, bacteria of, 160
    disinfection, 161; 265
Sleeping sickness, 170
Slides, forceps for, 27
    glass, 27
Small-pox, bacteria found in, 437
    cytoryctus variolæ, 422
    inoculation of, 10
    vaccination for, 200
Smear-culture, 80
    preparations, 26
Smegma bacilli, 33, 163
Snake-venom, 189
Sodium hydroxide, for neutralization of culture media, 64, 65, 66
Soft chancre, bacillus of (see Bacillus of soft chancre)
Soil, bacteria of, 135, 176
Solutions, normal, 66
Species of bacteria, 117
Spirilla in the mouth, 161
    for class demonstration, 114
    in water, 403
    S. dentium, 274
    S. milleri, 326
    S. plicatile, S. rugula, S. volutans, 274
    S. sputigenum, 274
    vibrio aquatiles, vibrio Schuylkiliensis, 403
Spirillum, definition, 4, 118, 120
    choleræ Asiatici, accidental infection of laboratory workers, 395
        description of, morphology, cultural characters, pathogenesis, 317
        detection of, in feces, 322
        diagnosis of, 322
        disseminated through water, 145
        in milk, 152
        killed by the hydrochloric acid of the gastric juice, 164, 393
        pathogenic properties for experiment animals, 393
        portals of entry into the body, 395
        reported detection of, in water, 145
        vaccine for, 394
Spirillum, dentium, 274
    of Deneke, 402
    of Finkler and Prior, 401
    of Metchnikoff, 400
    of Miller, 402
    of Obermeier, 404
    of Vincent, 275
    plicatile, 274
    relapsing fever, 404
    rubrum, 274
    rugula, 274
    sputigenum, 274
    tyrogenum, 402
    undula, 274
    volutans, 274
Spirochæta, definition, 120
    dentium, 274
    Obermeieri, 404
    of syphilis, 405
    pallida, 405
    plicatile, 274
    refringens, 406
Splenic fever (see Anthrax)
    puncture in typhoid fever, 378
Spontaneous generation, 3, 13
Spores arresting and resisting forms of bacteria, 3
    discovery of, by Cohn, 14
    reproduction of bacteria by, 122
    arthro, 123
    compared with vegetative cells, 122
    definition of, 3
    discovery of, 14
    endogenous, 122
    of moulds, 280
    of the malarial parasite, 416, 418
    resistance to heat and other germicides, 123
    staining, 48
Sporotricha or sporothrix, 232
Sputum, disinfection of, 34, 259
    method of making preparations of, 34, 108
    rules for collection of, 34, 107
    staining of, 34
Stab-culture, 78
Staining anilin-oil, water solution, 30
    bacteria in tissues, 37, 41
    blood, 45
        Hasting's method, 46
        Nocht's, Goldboron's, Wright's, 47
    capsules, 49
        Hiss' method, Welch's, 49

Staining, cover-glass preparation, 29
  diphtheria bacillus, 338
  flagella, 50
  Gabbett's solution, 35
  gonococcus, 311
  Gram's method, 31
    bacteria which stain by, 32
  Gram-Weigert method, 41
  malarial parasite, 45
  neuclei of tissues, 44
  sections, 41
  spores, 48
  tubercle bacillus in milk, 33
    in sputum, 35, 108
    in tissue, 41
Stalactite growth of plague bacillus, 321
Staphylococcus cereus albus, cereus flavus, 284
  definition, 118
  epidermidis albus in milk, 152
    in normal skin, 161, 263
    in suppuration, 284
    morphology, cultural character, pathogenesis, 294
  pyogenes albus in cystitis, 290
      in milk, 152
      in normal skin, 161
      in secondary infection, 287
      in suppuration, 284
      in wounds, 286
      morphology, cultural characters, pathogenesis, 294
    aureus in cystitis, 290
      in milk, 152
      in normal skin, 161
      in secondary infection, 287
      in suppuration, 284
      in wounds, 286
      morphology, cultural characters, pathogenesis, 291
    citreus, 284
      in cystitis, 290
      in milk, 152
      in normal skin, 161
      in secondary infection, 287
      in suppuration, 284
      in wounds, 286
Steam sterilization, 55
  (in contagious cases), 260
  (on animals), 104
Stegomyia, 154, 336
Sterilization, 55
  at autopsies (on animals), 104
    (in contagious diseases), 260
Sterilization, by boiling for various purposes, 55
    for drinking water, 142
    for surgical instruments, 266
  by filtration, 62
  by pasteurization, 60
  by steam, 55
  by the autoclave, 60
  by the naked flame, 53
  fractional, 55
  hot-air, 53
  intermittent, 55
  methods of testing efficacy of, 115
  of blood-serum, 59, 74
  of brushes used in surgery, 266
  of culture-media, blood-serum, 74
    by filtration, 64
    by live steam, 55
    by pasteurization, 60
    in autoclave, 60
  of cultures, 96, 116
  of dressings, 265
  of glassware, 53
  of gloves, rubber, 265
  of hands, 264
  of hypodermic solutions, 266
  of ligatures, 265
  of milk in infant feeding, 153
  of platinum needle, 79
  of steam, 51, 55
  of surgical instruments, 266
  of test-tubes, 76
  of water, 142
Sterilizer, Arnold, 56
  hot-air, 53
  Koch, 55
  Massachusetts Board of Health, 58
  steam, 55
Sternberg's bulbs, 110
  determination thermal death-point of bacteria, 125
Stewart's forceps, 26
Stick-culture (see Stab-culture)
Stitch-abscesses, 266
Stomach, bacteria of, 164
Stools, disinfection, 259
Straus's method for diagnosis of glanders, 365
Streak cultures, 80
Streptococcus brevis, 295
  definition, 118
  injection of cultures of, for cure of sarcoma, 299
  lanceolatus (see Micrococcus lanceolatus)

Streptococcus, longus, 296
 of erysipelas, 300
 polyvalent serum for, 298
 pyogenes (see also Suppuration), 294
  endotoxin of, 190
  in suppuration, 284
  morphology, cultural characters, pathogenesis, 294
  secondary infection with, 287
 serum, 247
Streptothrix, 228, 276, 369
 actinomyces, 366
 cuniculi, 277
Stropping knives, 40
Substance sensibilisatrice, 226
Sugar-free bouillon, 67
Sugars in culture-media, 67, 71
Sulphur dioxide, use in disinfection, 209, 254
Sunlight, influence on bacteria, 247
Suppuration, 14, 282
Surgical disinfection, 262
Surra, 409
Swarming islands of B. proteus, 263
Swine erysipelas, vaccine for, 202
Symptomatic anthrax, 202
Symptomatic anthrax, production of immunity in, 202
Syntoxoid, 223
Syntoxonoid, 224
Syphilis, various organisms in, 171
 spirochætæ in, 405
Systematic study of species of bacteria, 112

Teaching bacteriology, suggestions for, 113
Teeth, bacteria of, 162
Terminal infections, 183
Test-tubes inoculation of, 78
 kind best adapted to bacteriological work, 76
 manner of filling with culture-media, 77
  of holding, 78
 plugs for, 76
 sealing of, for anaerobic cultures, 91
  to prevent drying of the medium, 86
 sterilization of, 77
Tetanus antitoxin, principles involved in the production of, 209, 331
Tetanus bacillus, 84, 122, 269
  in soil, 135
  isolation of cultures by heat, 94
  morphology, cultural characters, pathogenesis, 327
 toxin, 162
  different in action from poisons, 188
  agents distructive to, 330
  extracellular character, 187
Tetrad, definition, 119
Texas fever, 177
Thermal death-point of bacteria, determination, 125
Thermophilic bacteria, 125
Thermostat (see Gas-regulator)
Thiothrix, 277
Thrush, 280
Thymol, 107
Tinea favosa, trichophytina, 281
Tissues, fixation and hardening, 37
 staining bacteria in, 37, 41
Titration of culture-media, 65
Toxemia, 184
Toxin, definition, 187
 endo-, 200
 of diphtheria and of tetanus, extracellular nature, 188
 of diphtheria mixed with antitoxin, 223
 potency of 189,
 resemblance to ricine, 209
Toxins as end-products of bacterial growth, 129
 extracellular, intracellular, 187, 200
 in production of antitoxin, 209
 necrosis produced by, 189
 potency of, 189
 spectra of, 224
Toxoid, 225
Toxon, 225
Toxonoid, 225
Toxophore group of the amboceptor, 219
Trichophyton, cultivation, 76
Trypanasoma brucei, dimorphon, equinum, equiperdum, evansi, gambiensis, theileri, 410
 of nagana, 410
 of surra, 410
Trypanosomes, 407
 cultivation of, 408
 description of, 408
Tsetse-fly disease, in surra, 77, 409

- Tubercle bacillus (see Bacillus tuberculosis) gray, miliary, yellow, 358
  - structure, 356
- Tuberculin, method of preparation, 360
  - R, 361
- Tuberculosis, 356
  - acute miliary, 358
  - bovine, avenues of infections in, 359
  - cross-infection of human beings and of cattle, 355
  - diagnosis by agglutination test, 360
    - by cover-glass preparations, 33
    - by culture, 350
    - by tuberculin test, 36
  - frequency, 151, 356
  - immunity, from, 362
  - infection from milk of, 151
  - infection of infants, 151
  - infection of meat of cattle suffering from, 152
  - of birds, 362
  - organs affected by, 358
  - pseudo-, 362
  - spread of, in the body, 358
  - tuberculin diagnosis of, 361
- Transmission through placenta, 173
- Typhoid fever bacillus (see Typhi abdominalis)
- Typhus fever, 170
- Tyrosin, produced by bacteria, 129
- Tyrotoxicon, 153

- Ultramicroscopic organisms, 171
- Unit, immunity, 348
- Urea, decomposition by bacteria, 129
- Urethra, bacteria, 164
- Urethritis, gonorrheal, 312
- Urinary bladder, bacteria of (see also Cystitis), 160
- Urine, disinfection, 259
  - preservation of samples of, 107
  - serum-agar, for cultivation of gonococcus, 313
  - typhoid bacilli in, 378
- Uterus, bacteria of, 160

- Vaccination, 11, 171
  - tetanus following, 329
- Vaccines, bacterial, 16, 201
  - black-leg, 202
  - cholera, 394
  - cow-pox, 200
  - hydrophobia, 203
- Vaccines, plague, 202
  - streptococcus, 298
  - swine erysipelas, 202
- Vaccinia, bacteria in, protozoa in, 421
- Vagina, bacteria of, 162
- Vaginitis, gonorrheal, 314
- Van Ermengem's method for staining flagella, 51
- Vegetative forms of bacteria, 122
- Venom of snakes, 189
- Vibro aquatilis, 403
  - Berolinensis, 402
  - definition, 120
  - Metchnikovii, 400
  - proteus, 401
  - rugula, 274
  - Schuylkiliensis, 403
- *Vibrion septique* (see Bacillus œdematis maligni)
- Villemin, first to produce tuberculosis by inoculating animals, 12
- Vincent, bacillus of, 275
- Vinegar, bacteria in, 5
- Violet, gentian-, aniline-oil, 30
  - aqueous, 23
  - methyl-, 251
- Virulence of bacteria, effect of artificial cultivation on, 82
  - influence of moisture on, 126
  - variability due to sundry causes, 182

- Warmth, relation to growth of bacteria, 125
- Water, B. coli communis in, 146
  - B. typhosus in, 145
  - bacteria of, 139
    - conveyed by, 139
- Water, collection of samples for examination, 142
  - detection of pathogenic bacteria in, 145
  - estimation of the number of bacteria in, 143
  - filtration, 63, 140
  - ground-, 139
  - infections carried by, 139
  - pathogenic bacteria in, 139, 145
  - purification by ozone, 142
  - recommendations of American Public Health Association, 147
  - self-purification, 139
  - spirilla in, 114, 274, 403
  - S. choleræ Asiatici in, 145

Water, sterilization of, 142
  storage of, 140
  transmission of typhoid fever, of cholera, of dysentery by, 176
  transportation of, for examination, 142
  typhoid fever bacilli in, 145
  well, contamination of, 139
Watery solutions of aniline dyes, 28
Weigert's stain for fibrin and bacteria, 42
Welch's stain for capsules, 49
Whooping-cough, 171
Widal's serum-test for typhoid fever, 376
Wire baskets, 77
  platinum, 22
  silver, 266
Wolffhügel plate, 144
Wool-sorter's disease, 137, 176, 334
Wounds, infected, 265, 181, 286
Wright's stain for blood, 47
  method for anaerobes, 91
Wurtz's culture-medium, 371
Wurzelbacillus (see Bacillus mycoides)

Xerosis bacillus, 343
X-rays, 247
Xylol, 38, 41

Yeasts, description of, 273
  for class work, 115
  in air, 137
Yellow fever, bacteria not the cause of, 424
  conveyed by mosquito, 440
  destruction of mosquito of, by sulphur dioxide, 255
    by petroleum, 258
  filterable virus, 172
  microörganism unknown, 170
  tubercle, 358
Yersin's serum for plague, 324

Ziehl's carbol-fuchsin, 34
Zinc chloride, sulphate, 258
Zoöglœa, 122
Zomotoxic group of the amboceptor, 222
Zymophore group of the amboceptor, 222

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